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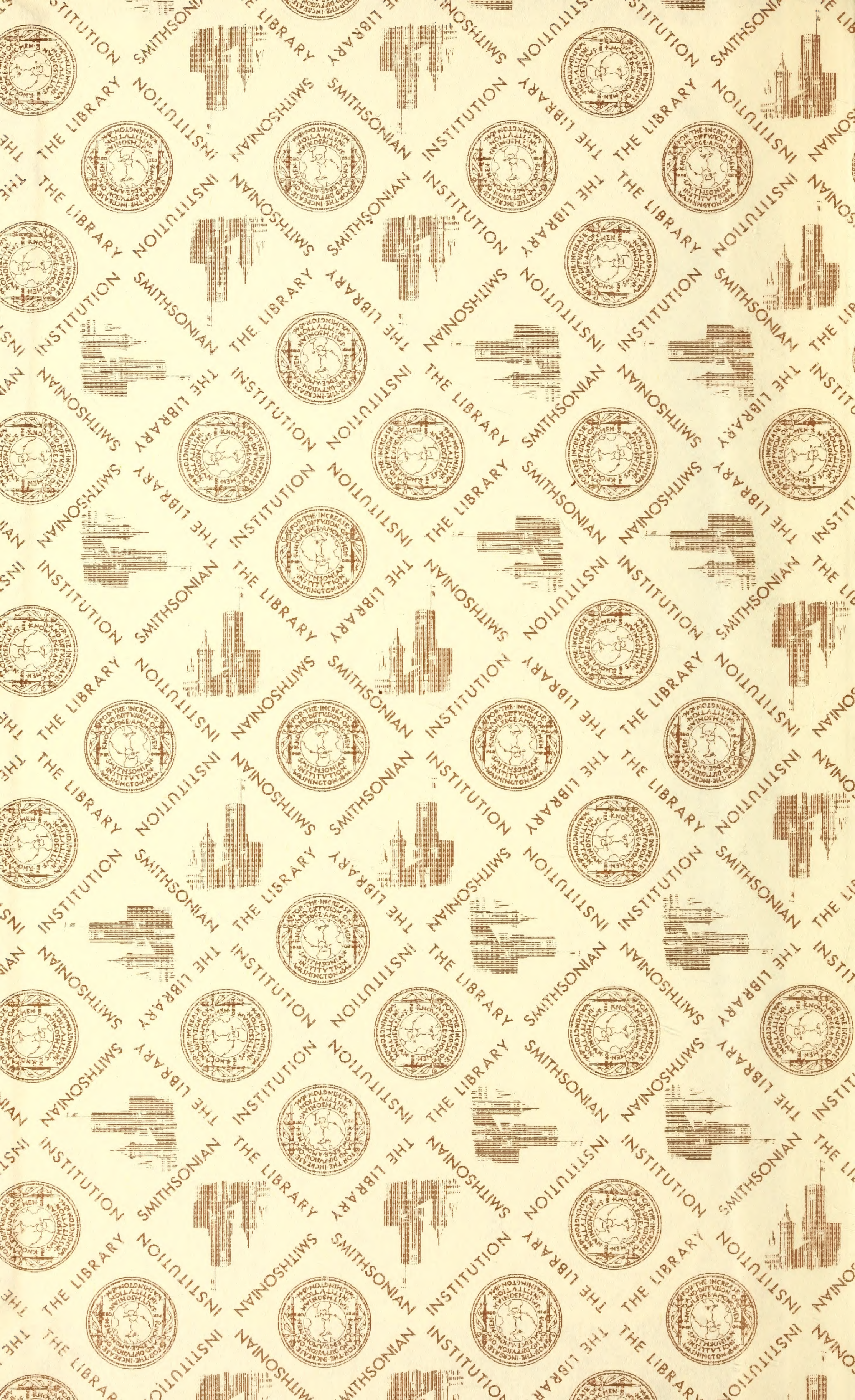
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AMERICAN JOURNAL OF BOTANY

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ERRATA

Plate VI, Number 2, for Fig. *13*, read 12.
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INTRODUCTORY

With this issue the American Journal of Botany starts upon its career with the support of the Botanical Society of America and the Brooklyn Botanic Garden. That there is field for a new botanical journal, no one acquainted with the progress of botany in America can question. Within the past twenty-five years the avenues for publication in this field have enormously increased, especially in Government territory. But great as it is, this increase of means has not kept pace with production. The result has been that our established botanical journals in America are over-stocked with manuscripts waiting their turn, our colleges and universities are making outlets for their own production, and foreign journals have their courtesy and capacity taxed by the offers of American contributors. All three of the conditions just named are undesirable: an author does not like to wait a year or more for the appearance of his paper, the multiplication of small periodicals by colleges and universities is a vexation to research, and it is neither just to ourselves nor kind to our colleagues of other lands to ask them to give large printing space to our contributions.

Thus it is certain that our new journal does not enter the field with keen competition. Matter for its printing is offered with outstretched hands. This lack of competition might be thought of as an unfortunate circumstance; but rather it can be made the opportunity for a high order of excellence to be adopted and maintained for the papers accepted for publication. The modern scientific journal has the duty not only of serving its contributors with space for publication, but also of serving its readers, as far as possible, with reading matter well put together and narrative concisely expressed.

The field of the AMERICAN JOURNAL OF BOTANY must be as wide as the whole science, for it is to serve the interests of organizations whose members come from all quarters.

The AMERICAN JOURNAL OF BOTANY is not blinded as to the benefit it is to bring to its science. It realizes that its five hundred to six hundred pages will afford but temporary relief for the ever-increasing congestion. It is to be hoped that the same organizations which have enabled long cherished plans to be brought to fruition in the establishment of this journal may be able to find the means from time to time to increase their capacity for publication, and thus to aid in offering to botanical research in America facilities proportionate to those enjoyed in Europe.

F. C. NEWCOMBE.

THE DEVELOPMENT OF AGARICUS ARVENSIS AND A. COMTULUS[1]

Geo. F. Atkinson

The development of *Agaricus campestris*[2] from the very young and undifferentiated carpophore was published in 1906. In 1905 young carpophores of several species of agarics in different stages of development were collected in the forests of the Jura Mountains in the vicinity of Pontarlier, France, during a three weeks' sojourn at that place. Among these were *Agaricus arvensis* Fr., and *Ag. comtulus* Fr.

MATERIAL AND VERIFICATION OF SPECIES

The material for the study of *Agaricus arvensis* was collected in a dense spruce forest, to the north of Pontarlier, maintained by the city government. The young carpophores were found in the forest mold in connection with the mycelium of a large plant which was not quite mature, *i. e.*, not fully expanded. The large immature individual was taken to my provisional laboratory in the hotel, photographed before the rupture of the veil, and then kept for a day or two for photographing in the fully expanded state. There may be some question as to whether this plant is *Agaricus arvensis* or *Agaricus xanthodermus* Genev. (= *Ag. flavescens* Roze, not *Ag. flavescens* Gillet). Other plants collected in the same forest, and communicated to M. Emile Boudier, of Montmorency, near Paris, were determined as *Ag. xanthodermus*. The single plant from the same mycelium as the young carpophores could not well be sent to M. Boudier since it was immature when collected and it was desirable to keep it until expanded for the purpose of photographing it.

The important characters of the plant noted at the time are as follows: Pileus and stem pure white, pileus smooth or with very fine appressed scales. Veil thick, ample, slightly cracked radially at the margin and with regular, angular scales on the under side of the margin. The flesh remained unchanged where bruised, and the surface of the pileus did not yellow to the touch, perhaps due to the fact that the plant was collected young and then kept for a few days under unnatural conditions. The flesh of the stem was not yellow at the base which is said to be a character of *Ag. xanthodermus*. There was a slight odor of almonds, which is present, however, in several species of *Agaricus*.

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[1] Investigation prosecuted with the aid of a grant from the Botanical Society of America in 1905.

[2] Atkinson, Geo. F. The development of *Agaricus campestris.* Bot. Gaz. **42**: 241–264 *pls. 7–12.* 1906.

This forest form described above (no. 21054 of my collections) is quite different in appearance from the robust form which occurs in pastures in the Jura Mountains. It is almost impossible to obtain full grown forms of these robust specimens in the vicinity of Pontarlier, since they are much prized as edibles and the localities where they are known to occur are visited almost every day by collectors who take them in the young stage soon after their emergence from the ground. On a hillside of a pasture northeast of Pontarlier there is a large arc of a fairy ring which is probably several centuries old. If the circle of this arc were completed its diameter would be several kilometers. M. August Dornier, of Pontarlier, in whose house I lived during my stay in 1910, informed me that during the past 25 years he had collected *Agaricus arvensis* each year from this same arc of a fairy ring. It is a long arc with a slight but gradual curvature, and is quite conspicuous on the hillside because of the dark green color of the grass.

I collected several young plants from this ring on August 8, 1910. They were quite robust and were just beginning to expand. They were carried to my room, transplanted into pots of earth, covered with a bell jar and photographed August 10 and 11. The removal from the earth checked their development.

Although they opened so that fair photographs were obtained, the plants did not reach their full size. Notwithstanding this, the pileus of the largest measured 12 cm. in diameter and the stems 3–5 cm. thick. The pileus was white, then gray and very scaly at maturity with fibrous appressed scales, many of them upturned toward the margin. Bruises of the surface of pileus and stem change slightly to yellow or pale saffron. The veil is large, thick, very floccose below and radiately split into coarse scales toward the margin, those on the edge being tinged with gray. The stem is smooth above the annulus but very scaly below, with larger conic scales crowded near the base and often in concentric rings. In age these large scales become dull pale brown, while upward they grade into smaller and lighter colored ones, the uppermost, next the annulus being floccose and white. There was a faint odor and taste of almonds. M. Boudier, to whom specimens were sent, confirmed the identification as *Ag. arvensis*. It is therefore a peronate variety of *Agaricus arvensis* comparable with *Agaricus perrarus* Schulzer,[2] which is considered by some to be a form of *Agaricus augustus* Fr. Similar forms of *Agaricus subrufescens* Peck[3] are sometimes found, which perhaps are identical with *Ag. perrarus* Schulz. while the normal forms of *Ag. subrufescens* Pk. are very near to *Ag. augustus* Fr., and this *Ag. subrufescens*, perhaps, may be considered a slender, small-spored variety of *Ag. augustus*.

Another form intermediate between this strongly peronate form of *Ag. arvensis*, and the forest form (no. 21054), was found not far from the fairy ring described above. This (no. 24763) plant was growing in the edge of a small clump of bushes and small trees situated in the pasture, and was fully expanded. It was 20 cm. high, the pileus 15 cm. broad and the base of the stem 4 cm. thick. The pileus was white, smooth or with delicate appressed scales. The annulus was large, membranous, and the under surface with large regular scales near the margin, radiately arranged. The stem was smooth, at first white, then later slightly rufescent and minutely scaly over the upper part of the fusoid bulb or base. There was a slight odor and taste of almonds.

Agaricus arvensis is therefore quite variable in the Jura Mountains according to location and other conditions. A number of different varieties of *Agaricus arvensis* are recognized by some authors, though there will probably always be differences of opinion as to the limits of this and related species, because of their great variability, and because of different opinions as to the concept of species. But it seems quite reasonably certain that the material from which this study of development is made belongs to a forest form of *Agaricus arvensis*.

DEVELOPMENT OF AGARICUS ARVENSIS

Primordium of pileus, stem and hymenophore

Early differentiation of the interior of the carpophore.—Fruit bodies 2.5 mm. long by 2 mm. in diameter show a faint differentiation of the internal portion into pileus and stipe fundaments, but the peripheral portion of the carpophore stains more deeply. The hyphae of the peripheral portion have somewhat thicker walls and less protoplasmic content than those of the interior, *i. e.*, of the pileus and stem fundaments. The walls of these hyphae are well stained with the haematoxylin, and this portion contrasts rather clearly with the interior because it is more deeply stained and also because the meshes of the tissue are somewhat larger. The features which mark the differentiation of the internal portion into pileus and stem are the very slight indication of the annular gill cavity, the appearance of a constriction between the stem and pileus fundaments, and the abrupt narrowing of the internal portion, so that the pileus primordium appears as a small round projection from the broader basal portion, the primordium of the stem. The looser, more deeply staining tissue surrounding the pileus and stem primordium is fundamental tissue, the outer portion of which may be regarded as the "universal veil," though its inner limits cannot be clearly defined.

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[2] Verhand. K. K. Zool. bot. Ges. —: 493. 1879. See also Bresadola, J. Fungi Trid. **1**: 54. *pl. 89*. 1887.

[3] Described in 6th Rept. N. Y. State Mus. Nat. Hist. 25. 1893.

On the exterior the young carpophore does not yet show any constriction because the enveloping zone of fundamental tissue has not yet become subject to distortion from the internal changes of form. This condition exists until some time after the origin of the primordium of the hymenophore, when the expansion of the pileus becomes greater than the lateral expansion of the stem, and an external constriction is then evident.

The hyphae of the interior portion of the very young fruit body are 3–4.5μ in diameter, have very thin walls, and the rather scant cytoplasm alone appears to be stained.

Character of the pileus primordium.—The annular gill cavity is formed by the more rapid growth and extension of the surrounding tissue. It appears between the margin of the pileus and the stem fundaments. The absence of a more deeply staining central portion in the upper part of the young fruit body contrasts strongly with the condition in *Hypholoma* described by Miss Allen,[4] in *Lepiota clypeolaria*,[5] and with the condition described by Fayod[6] as the rule for the Agaricaceae. It agrees with the condition found by myself in *Agaricus*

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[4] Allen, Caroline L. The development of some species of *Hypholoma*. Ann. Myc. **4**: 387–394. *pls. 5–7.* 1906.

[5] *Lepiota clypeolaria* will be described in another paper.

[6] Prodrome d'une histoire naturelle des Agaricinés. Ann. Sci. Nat. Bot. VII, **9**: 181–411. *pls. 6, 7.* 1889.

campestris,[7] except that in the specimens of the latter species studied there was no morphological differentiation of the fundamental tissue indicating the pileus and stem fundaments distinct from the surrounding fundamental tissues, as in *Ag. arvensis,* until some time after the first evidence of the hymenophore primordium. In the young fruit bodies of *Agaricus campestris* examined, there appears an annular region of more deeply staining hyphae before any evidence of the annular cavity is seen, and just above the region where this is formed.

Median longitudinal sections of these very young carpophores of *Agaricus arvensis* show that the hyphae in the pileus primordium have in general a radiate direction. But the central ones show this character only in a slight degree, the hyphae are much interwoven and over the central area where the pileus primordium passes over into the external fundamental tissue they are intricately interwoven, no radial direction is evident and they show no evidence of specially active growth.

The lateral hyphae, however, show distinctly a radial direction and curve outward at the same time somewhat like the lateral stalks in a sheaf. The outer and lower ones curve more strongly, and the terminations of the lower ones curve downward next the outer fundamental tissue and directly above the looser mesh where the annular gill cavity is forming. These hyphae stain more deeply than the remaining elements of the pileus primordium, but the contrast is not very striking, and they lie very close to the external fundamental tissue which is deeply stained. Consequently this primordium of the hymenophore and pileus margin is not differentiated in the photograph of the entire section, figure 1. But in figure 8 more highly magnified, it can be seen that just above the more open mesh there is a narrow area next the external fundamental tissue where the hyphae compacted together have a downward direction. The external fundamental tissue presents a very distinct texture since the walls of the hyphae are thick and the mesh is open though not so open as that where the gill cavity is forming.

The primordia of the hymenophore and of the pileus margin are merged in the early stages.—In slightly older stages, as shown in figure 2, the gill "slit" is distinct though narrow, and the primordium of the hymenophore is very distinct, not only by the deep stain of the tissue, but by the parallel course of the crowded hyphae, which now form a more extensive area. The primordium of the pileus margin into which the hymenophore merges is also distinct. The hyphae are parallel and epinastic. It can be seen here, and in still older carpophores, that the radial and parallel arrangement of the hyphae, and their deeper stain, progresses centripetally, *i. e.*, toward the center of the upper surface of the young pileus. This is brought about because successive areas in the same direction in the pileus primordium gradually assume active growth, new hyphae are formed in abundance which extend radially, and curve downward under the influence of epinasty. The advance hyphae penetrate through the fundamental tissue. Some of them can be seen to grow into the inner portion of the external fundamental tissue.

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[7] Atkinson, Geo. F. The development of *Agaricus campestris.* Bot. Gaz. **42**: 241–264. *pls. 7–12.* 1906.

Character of the hyphae in the young hymenophore primordium.—Here, as described for *Agaricus campestris*, many of the earlier hyphae of the young hymenophore primordium are very slender and sharp-pointed. This form permits them more easily to make their way through the tissue until they reach the annular cavity. When the sharp-pointed hyphae are exposed in the annular cavity, their form soon changes, or the new elements developed are different in form. The hyphae are now short, blunt at their free ends and form a very compact palisade layer. As centrifugal growth of the pileus continues at the margin, the new elements arise in the same order as the gill cavity is extended. The newer elements growing into the cavity are sharp-pointed and often crowded into an uneven palisade layer, but the transition to the blunt palisade cells is soon effected.

In figures 1–3, the youngest carpophores studied, the internal portion immediately below the constriction, and the annular cavity, is more deeply stained than the pileus primordium and the basal part of the stem. This activity of the hyphae in this region seems to be connected with the primordial differentiation of the stem. This can be traced in the older carpophores as seen in figures 2, 3 and 5, where the demarcation of the stem surface gradually appears. The stem in the very young fruit bodies is thus seen to be very short, its breadth exceeding its length for some time. The tissue marking the outline of the stem is thus more active and stains more deeply than that of the pileus, except in the immediate region of the hymenophore, and the margin of the pileus, where growth and the formation of new hyphal elements is necessarily active.

It is interesting to observe that during the early growth of the young hymenophore, and of the gill cavity, their outline rises from the point next the stem at an oblique angle outward and upward. Epinastic growth of the hyphae in the margin of the pileus begins early so that the hymenophore at this point curves outward and downward over the upper angle of the gill "slit." As the fruit body enlarges, continued epinasty tends to move the whole pileus margin downward while the extreme margin is inrolled. At the same time elongation of the stem begins which tends to carry the inner angle of the gill slit and the hymenophore upward, so that the angle at which they formerly stood is reversed and they extend outward and downward.

Origin of the partial veil.—Since the hymenophore is endogenous in origin, the partial veil originates from the fundamental tissue lying outside the annular cavity and is not clearly separated from the universal veil. It is thus connected with the margin of the pileus and the surface of the stem. This fundament of the annulus, or partial veil, increases in extent by tension resulting from the expansion of the pileus and stem, and also by growth of the elements, which growth, however, does not keep pace with that of the surrounding parts. In the older fruit bodies, as shown in figures 5 and 6, this veil is duplex in structure. The lower portion lying next the stem surface has a looser texture, is the principal aerating tissue, is derived from fundamental tissue and increases by growth of its elements. The upper portion lying next the gill cavity is connected directly with the margin of the pileus. It originates partly from fundamental tissue and partly by growth from the margin of the pileus, the growth from the pileus probably forming the denser portion. The lower looser portion is torn off from the surface of the stem during further growth and expansion of the plant, and provides the looser lower portion of the duplex veil characteristic of *Agaricus arvensis* and some of the other species of *Agaricus*, like *Ag. augustus* Fr., etc. Its looser texture would permit of its being torn into angular, often radiating areas or patches, so striking a feature of the duplex veil of these species. In very robust specimens the increase in the fundamental tissue between the partial veil and stem appears sometimes to be very great and this very likely provides the looser tissue which forms the floccose scales on the stem below the annulus in the peronate forms.

DEVELOPMENT OF AGARICUS COMTULUS

Primordia of pileus, hymenophore and stem.—The youngest fruit body of *Agaricus comtulus* Fr. sectioned was 1.25 mm. long by .75 mm. in diameter. This shows the earliest stages of the primordium of pileus, stem and hymenophore, before there is any indication of the annular gill cavity. In longitudinal section the primordium of the hymenophore appears as two dense, deeply staining, rounded areas, symmetrically located on either side of the axis and some distance below the surface, showing that it is also endogenous in origin. The pileus primordium above is indicated by a very faintly staining dome-shaped area. Below, the stem primordium is indicated by a more densely staining area, distinct in this respect from the lighter staining basal area in which the tissue is of a looser texture.

Character of the external zone of the young carpophore.—Above the pileus primordium, and lateral to the hymenophore, the tissue is of a much looser texture, the hyphae in general having a radial arrangement but flexuous and intricately interwoven with adjacent ones forming a loose mesh work, not at all comparable to the palisade layer of radial hyphae enveloping the upper part of the carpophore in *Lepiota clypeolaria*, *Armillaria mellea*, etc.

Later stages of organization.—In the next stage examined the fruit body was 5 × 3 mm. The fundamental parts of the fruit body are well advanced and the external constriction marks the limit of pileus and stem. The gill cavity and hymenophore are already evident (figure 10). The rudimentary hymenophore is furnished with the palisade layer of blunt cells. The tissue which forms the partial veil, lying between the annular gill cavity and the outside is already of much looser texture, the lower portion of a much more open mesh, while the upper portion next the hymenophore or gill cavity is more dense as described above for *Ag. arvensis*.

In figure 11 is represented a much older fruit body where gill formation has already reached quite an advanced stage. The outline of the stem surface is very clearly shown. The duplex character of the veil is very striking with its looser texture below and the denser portion above. The further stages of its development are as usual.

THE UNIVERSAL AND PARTIAL VEILS IN AGARICUS

In the three species of *Agaricus*, which I have thus far studied, the hymenophore is of deeper origin than that of *Psalliota rubella*

(*Agaricus rubellus* Gillet) as figured by Fayod[8] (l. c., *pl. 7.* fig. 4) which he places in his subangiocarpous type ("*type subangiocarp.*" pp. 284, 285) to which he says the majority of the species of *Psalliota* (*Agaricus* as limited here) probably belong. In this type, according to Fayod, the universal veil is not differentiated from the cuticle of the pileus but remains concrete with the pileus as stated by Fries[9] for the species of his Tribe *Lepiota.* In the subangiocarpous type Fayod interprets the universal veil (voile generale) to include the partial veil, the outer layer of the carpophore continuous with the partial veil, together with the fundamental tissue which separates from the stipe. It would include the outer zone of the mature pileus, since the universal veil, according to this interpretation, does not separate from the pileus but remains in intimate union with it.

A primary universal veil in Agaricus campestris.—In *Agaricus campestris* there is often formed a well differentiated, but delicate and floccose, universal veil,[10] quite distinct from a very young stage, which at maturity separates distinctly from the surface of the pileus. It is as strongly developed as the universal veil which is present in *Pholiota caperata* on which Karsten based his genus *Rozites.*[11] But in this species there is a definite pileus cuticle or cortex of specialized cells, forming a clear and distinct differentiation from the universal veil. In *Agaricus* the cuticle of the pileus is fibrous and not specialized. In other species of *Agaricus* a similar delicate universal veil is sometimes present. What I have heretofore regarded as the universal veil in *Agaricus campestris* is this outer layer of loose fundamental tissue. It is a primary universal veil, evident on the young carpophore, and which can be seen in sections at later stages as a thin loose layer enveloping pileus and partial veil, though not separated so cleanly from the carpophore as in the genus *Amanita* where the universal veil is usually much stouter, and not strictly homologous with the freed portion in *Agaricus campestris.*

Organization of pileus surface.—In no one of the three species of *Agaricus* which I have studied does the pileus surface, during its early organization, stand out so distinctly from the enveloping tissue as is shown in the illustration of *Agaricus rubellus* by Fayod.[12] Although, in some cases it appears that the primordium of the pileus, in the form of an internal area, central in the upper part of the carpophore primordium, may be distinguished from the surrounding tissue, slightly before or simultaneous with the earliest appearance of the hymenophore primordium (see figure 4) by the more active growth of the hyphae, and consequently the deeper staining of this area, the hyphae are intricately interwoven and show no organization into a definite structure. The earliest differentiation which indicates the organization of a structure, in the three species which I have studied (*A. campestris, arvensis* and *comtulus*), is marginal, and is the primordium of the hymenophore. Since it is impossible in very young stages to clearly distinguish between the hymenophore and pileus margin, this early marginal growth may share in the organization of both structures. Epinastic growth is first manifested in this marginal region, and, simultaneous with the progressive development of the hymenophore, epinasty is more and more marked.

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[8] Prodrome d'une histoire naturelle des Agaricinés. Ann. Sci. Nat. Bot. VII. **9**: 181–411. *pls. 6, 7.* 1899.

[9] Syst. Myc. **1**: 19. 1821.

[10] Atkinson, Geo. F. The development of *Agaricus campestris.* Bot. Gaz. **42**: 241–264. *pls. 7–12.* 1906.

[11] Karsten, P. A. Rysslands, Finlands och Skandinavikar Holfäns Hattsvampar. **1**: 290. 1879.

Organization of the primordial surface of the pileus.—The organization of the pileus structure, judged by the radial and epinastic growth of the hyphae, and the clearness with which it can be differentiated from the external fundamental tissue, is marginal also. While it is not separated from the external fundamental tissue, the parallel, radial and epinastic direction of the hyphae enables one to distinguish it quite clearly from the interwoven texture of the external fundamental tissue. From this point the organization of the pileus cortex seems to proceed to some extent in a centripetal direction, but the region over the center is very limited in such young specimens and the hyphae of course do not grow from the marginal area toward the center. Successive areas of the primorial tissue of the young pileus surface, in a centripetal direction, organize new and abundant hyphae which grow in a radial direction, and become curved downward by epinasty. The subsequent development of the pileus is for the most part centrifugal.

Fundament of the universal veil.—In figures 1–4 of *Agaricus arvensis*, and figures 7 and 10 of *Ag. comtulus*, external to the fundaments of the pileus and stem is a broad zone of fundamental tissue characterized by a more open mesh. It has already been noted that this tissue is composed of thick-walled hyphae quite distinct from the thin-walled hyphae of the denser inner tissue, though at the border between the two there is more or less a merging of both elements. This zone of tissue is homologous with that in *Amanitopsis vaginata* which forms the volva or universal veil. In the young carpophores of *Amanitopsis vaginata* there is a similar loose-meshed tissue surrounding the primordium of pileus and stem which merges gradually into the denser tissue within, and which only at a later period is separated as the volva, by a zone of gelatinizing hyphae. This universal veil while still undifferentiated may be called the *blematogen*,[13] or *blematogen layer*, yet unfashioned as a volva.

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[12] Also the structure of the universal veil as figured by Fayod for *Agaricus rubellus* is very different from anything which I have seen in the species of *Agaricus* which I have studied.

Relation of partial veil, "universal veil" (blematogen layer) and primary universal veil.—When the annular gill cavity is formed in *Agaricus*, it separates from the stem at this point, the fundament of the marginal, or partial veil. Since the universal veil or blematogen layer at this stage of development envelops the young carpophore, the question arises as to the relation existing between marginal or partial veil and the universal veil. Is the marginal veil merely a section of the universal veil as Fayod states, or is it a structure partly *sui generis*, and partly consisting of the universal veil which is external at this region? These questions are difficult to answer precisely, since the universal veil, or blematogen layer, in the genus *Agaricus*, and in a number of other genera, is never clearly differentiated in structure from the carpophore as it is in the Amanitae. The delicate, floccose, loose scales sometimes present on the expanding and mature plants as shown in *Agaricus campestris*,[14] may be regarded as the primary universal veil, the *protoblema*, or *protoblem*.[15] Beneath this primary veil or protoblem, in the young carpophore of *Agaricus campestris*, is the zone of loose-meshed tissue homologous with the undifferentiated universal veil, or blematogen layer in *Agaricus arvensis*, *Ag. comtulus*, and in *Amanitopsis vaginata* just described. But since, in these species of *Agaricus*, no distinct cuticle or cortex of the pileus of a definite cellular structure, markedly different from that of the blematogen is developed, which releases the universal veil as is said to be the case in Fayod's angiocarpous type (l. c., p. 286), nor is released by the gelatinization of an intermediate zone as in the *Amanitae*, it may have no very great taxonomic significance. It is fundamental tissue of limited extent, the outer layers are often gradually and irregularly broken down and partly exfoliated as the plant matures. Where it is quite distinct at maturity there occurs considerable increase of its elements during early stages of growth. An inner zone, or in some cases perhaps nearly all of it, remains in coalescence with the undifferentiated surface of the pileus. But when it is of the character presented by *Agaricus arvensis* (figures 1–4) and *comtulus* (figures 7 and 10), consisting of thick-walled hyphae, further growth of its elements is probably not extensive. In such cases the outer portion is sloughed off or exfoliated as shown in figures 5, 6 and 9, leaving the surface of the young pileus more or less eroded for a time from an irregular cleavage plane through the zone of the universal veil.

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[13] βλημα = cover; γενής = producing.

[14] See Plate 10, and Plate 12, figure 18, in Atkinson, Geo. F. The development of *Agaricus campestris*. Bot. Gaz. **42**: 241–264. *pls. 7–12.* 1906.

[15] πρῶτος = first; βλημα = cover.

Since no definite boundary between the universal veil and pileus can be found in these examples, it appears to me, that, in the genus *Agaricus*, the external fundamental tissue below the pileus fundament is not always separated from the fundament of the partial veil, but gradually passes over into it, so that the partial veil really has a duplex structure at a very early stage, different from the duplex condition which appears later due to growth from the margin of the pileus and from the surface of the stem. The innermost portion of it is continuous with the margin of the pileus tissue from a very early stage. Further development of this fundamental tissue, forming the marginal veil, takes place after its separation from the stem primordium by the annular gill cavity. The inner portion of it is also increased by continuation of the growth of threads from the margin of the pileus which seems to indicate rather clearly that the marginal or partial veil is not wholly made up of a section of the universal veil.

The "universal veil," or blematogen layer, then, in the species of *Agaricus* thus far studied by myself, one might say consists of a poorly defined external zone of fundamental tissue, not clearly differentiated even after the organization of the pileus, so that the inner portion is more or less concrete with the pileus and marginal veil, while the outer portion may entirely disappear or remain as loose fragments or delicate scales on the surface of the pileus, forming in some cases a slight external contingent of the marginal veil.

Where the external free contingent of the primary universal veil, or protoblem, is abundant, a distinct, though slight annulus may be formed at the base of the stem. This I have observed a few times in the case of *Agaricus campestris*.

Smith figures diagrammatically the structure of *Agaricus campestris*.[16] Since there is no discussion of the morphological characters of *Agaricus* it is difficult to understand what his conception is of the universal veil and marginal veil in this genus. He figures a universal veil (U.V.) which extends from the base of the stem up over the entire carpophore and also a small ring at the base of the stem as the lower remnant of the universal veil after expansion of the plant. In addition to this an inner veil, the partial veil is illustrated as a distinct structure which forms the annulus. In his diagnosis of *Psalliota* (l. c., p. 170), he says: "Veil universal, concrete with the cuticle of the pileus and forming an annulus on the stem." The annulus which he figures from the universal veil is at the base of the stem while the structure marked annulus (A.N.) is midway on the stem. In his characterization of the family *Agaricaceae* (l. c., p. 11) he clearly distinguishes between the "*primary or universal veil*" which forms the volva and fragments on the pileus, and the partial veil. "In some species a *secondary* or *partial* veil is also present in the earlier stages spreading from the upper part of the stem to the edge of the pileus. This veil is finally ruptured and partly persists as a ring or annulus (A) encircling the stem."

Cooke[17] says in his characterization of *Psalliota* (*Agaricus*), "Veil universal, concrete with the cuticle of the pileus, and fixed to the stem, forming a ring." In his Tribe *Psalliota* Fries[18] says "veil annuliform, subpersistent, strictly speaking partial," and adds that a rudiment of a subuniversal veil is present in some species among which is *Ag. campestris*. This "subuniversal veil" is probably what I have here termed the primary "universal veil," or protoblem. It appears that some authors regard the partial veil in *Agaricus* and some other genera merely as a part of the "universal veil," while others regard it as a distinct structure.

It does not seem worth while to inquire further into the literature, which is purely systematic, for the characterizations given are, in general, not based on studies of development from very young stages.

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[16] Smith, W. G. Synopsis of the British Basidiomycetes, fig. 42. 1908.

[17] Cooke, M. C. Handbook of British Fungi **1**: 136, 137. 1871.

[18] Fries, E. Syst. Myc. **1**: 280. 1821. "*velum annuliforme, subpersistens, proprie partiale.*"

Comparison with Lepiota.—It is worthy of note, however, that while several authors since Fries' time have stated that in *Agaricus* (*Psalliota*) the "universal veil" is concrete[19] with the cuticle of the pileus, Fries used this expression only in connection with his Tribe *Lepiota*.[20] Studies on the development of species of *Lepiota* may throw some light on the relation of the universal veil and partial veil in forms where the "universal veil" is not clearly separated from the pileus. I have studied the early stages of development in *Lepiota clypeolaria* and find a well-formed external layer, duplex in structure enveloping the entire carpophore except the extreme base. This may well be considered a "universal veil" or the fundament of one, a *blematogen layer* which does not become separated from the pileus. The portion of the partial veil lying between the margin of the young pileus and stem is differentiated later, and although the universal and partial veils never become clearly separated from each other, the position and structure of the universal veil is so characteristic in the young stages, that it seems reasonable from a purely morphological standpoint to recognize a "universal veil," or blematogen layer, and, in addition, a partial veil. A full account of these studies on *Lepiota clypeolaria* will be published in another paper.

In the species of *Agaricus* here considered, the structure which may be regarded as representing the "universal veil," or blematogen layer, is far less distinct than it is in *Lepiota clypeolaria*, though clearly as distinct as it is in the young carpophore of *Amanitopsis vaginata*. But since there is a poorly defined external layer of the fundamental tissue lying outside of the more or less parallel, radiately arranged hyphae which organize the surface or cuticle of the pileus, though not clearly separated from it, we may consider this layer as homologous with the "universal veil," a blematogen layer in *Agaricus* also. As interpreted here it is a thin layer covering the young pileus and partial veil. In the very young carpophores it is rather thick and prominent in comparison with the fundaments of pileus and stem. As the expansion of the young plant takes place the outer portion of it is torn into fragments which may be so scant as to be unrecognizable, or disappears, or may appear as small patches on the pileus. Some of the inner portion may remain in close connection with the surface of the pileus, and may be said therefore, to be concrete with it. But the evidence of the presence of a "universal veil" and its relation to the pileus is not so clear as in *Lepiota clypeolaria.*

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[19] See Cooke, M. C. Handbook of British Fungi, 136, 137. Fayod, V. Ann. Sci. Nat. Bot. VII, **9**: 181–411. *pls. 6, 7.* 1889.

[20] Syst. Myc. **1**: 19. 1821.

Comparison with the Amanitae.—In the Amanitae, "the universal veil," quite prominent even in the young carpophores, is still not precisely differentiated in *Amanitopsis vaginata,*[21] not more so than in the young carpophores of *Agaricus arvensis* and *Ag. comtulus.*

When the pileus surface is quite well organized in the *Amanitae* there is a zone of parallel hyphae between the pileus and universal veil which gelatinizes, or disintegrates in other ways, and separates the universal veil[22] clearly from the pileus. The position of the partial veil in the *Amanitae* is such that at no time is there any question as to its distinctness from the "universal veil," though the fundamental tissue from which it originates is in contact with that of the universal veil at an early stage of the carpophore. It must be remembered that in the primordium of the carpophore, the fundamental tissue, from which all parts of the plant arise, is continuous and undifferentiable, but this does not mean that when organized the stem is to be considered a part of the pileus.

Is there a primary universal veil, or protoblem, in A. arvensis and Ag. comtulus?—It is quite possible that, in the very young carpophores of *Agaricus arvensis* and *Ag. comtulus,* there is a primary universal veil, or protoblem, as in *Agaricus campestris,* and that it is represented in part by the thin exfoliated portions shown in figures 5, 6, 7, and 10. But in carpophores developing underneath the substratum, as these were, it is difficult to determine this point with precision. In specimens artificially grown, where the carpophores are exposed from a very young stage, as is often the case with *Agaricus campestris* in culture, it should not be a difficult question to settle.

Double margin of the partial veil.—In *Agaricus* as epinastic growth inrolls the margin of the pileus, the increase of the fundamental tissue of the partial veil often covers the external surface of the inrolled pileus margin. In such cases, as the pileus expands and the margin separates from the edge of the partial veil, the latter presents two edges. When the veil is quite tumid and not broad, and the margin of the pileus is very thick as in *Agaricus campestris* var. *edulis,*[23] or in

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[21] The development of *Amanitopsis vaginata* will be published in another paper.

[22] Now a *complete* or *finished veil,* or *teleoblema* (τελειος = complete or finished, βλημα = cover).

[23] Vittadini, G. Fung. Mang. 41. *pl. 6.* 1835.

Agaricus rodmani Peck[24] (which may be identical with the variety *edulis*), the annulus has a double edge. Since the lower edge slips off from the under surface of the pileus margin, it might be regarded by some as a volva. But in robust specimens of *Agaricus campestris* where the double margin of the annulus is often marked, there is frequently, in addition, a delicate ring at the base of the stem, the lower remnant of the primary universal veil or protoblem. The annulus with its duplex edge is situated above the base of the stem, and its lower limb is separated from the surface of the stem during expansion. These two facts in connection with what is known as to the considerable increase of the fundamental tissue from which the partial veil originates, argue against the volva nature of the lower limb of such an annulus, and also speak in favor of regarding the partial veil of *Agaricus* as distinct from the "universal veil" and not merely a section of it.

SUMMARY

1. In *Agaricus arvensis* the very young carpophores show an internal differentiation into a pileus and stem primordium, the pileus primordium appearing as a small rounded projection from the broad stem primordium, and there is a very slight constriction at the point of junction of the two. At the point of constriction the fundamental tissue presents a more open mesh indicating the tension from expansion of the surrounding parts which eventually forms the annular gill cavity. The fundaments of the pileus and stem at this stage stain faintly in contrast with the deep stain of the surrounding fundamental tissue. Nevertheless there can be discerned at the lower margin of the pileus fundament, just above the point where the annular gill cavity is to be formed, a small area of compact parallel hypae which form the fundament of the hymenophore and pileus margin.

2. Enveloping the primordia of the stem and pileus is a zone of fundamental tissue. The walls of the hyphae are thicker than those of the pileus and stem fundaments, and stain deeply but the mesh is more open. An outer, ill-definable zone of this represents the "universal veil," or blematogen layer, homologous with a similar layer in *Amanitopsis vaginata*.

3. The outline of the annular gill cavity, and the young hymenophore during the early stages, rises outwardly at an oblique angle from

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[24] 48th Rept. N. Y. State Mus. Nat. Hist. 137. 1897.

the stem. But later as the epinastic growth of the pileus becomes stronger, and the stem begins to elongate, the direction becomes reversed, and the outline of the gill cavity and the hymenophore extends outward and downward.

4. Distinct organization in the pileus first appears at the margin, indicated by the parallel growth of the hyphae, under the influence of epinasty. The surface of the pileus proper can here be distinguished from the external, intricately interwoven fundamental tissue which constitutes the "universal veil," or blematogen layer.

5. Stem organization can be observed in the more deeply staining portions of the stem fundament, as growth continues. At first it is broader than long, the outline of the stem cortex staining more deeply.

6. The marginal, or partial, veil is first differentiated by the appearance of the annular gill furrow. It extends from the margin of the pileus to near the base of the stem, and is covered externally by the "universal veil," usually with no well-formed line of demarcation between the two structures. The tissue of the partial veil increases greatly by growth of this inner fundamental tissue, by growth also from the margin of the pileus, which forms a part of the inner (upper) more dense portion of the veil. Abundant increase occurs also next the stem on the lower (outer) side of the partial veil. Thus is formed a duplex partial veil of much looser, more spongy structure below. As the plant expands the partial veil is at first torn from the lower surface of the stem. As it is stretched by the expansion of the pileus the looser, spongy, lower portion of the veil is separated from the pileus margin and surface of the stem and torn into the scales characteristic for the species, while the firmer upper (inner) portion is later separated from the margin of the pileus. In very robust specimens this loose tissue of the under surface of the annulus may be so abundant as to leave many scales on the stem giving it a peronate character.

7. In the very early stages of *Agaricus comtulus*, the first evidence of the differentiation into pileus and stem primordia is a dense, deeply staining, internal, annular area, appearing in longitudinal section as two deeply staining areas symmetrically disposed. This is the primordium of the hymenophore and probably also of the pileus margin. At the same time a less deeply staining dome-shaped area, the margin of which connects with the internal annular hymenophore primordium, or a general central area of larger extent, marks the differentiating pileus primordium. The stem fundament lies below.

8. External to the pileus primordium and the upper portion of the stem fundament is a zone of fundamental tissue with an open mesh. The hyphae in general have a strong radial direction but are clearly interwoven into an open-meshed plectenchyma. The surface is not marked by free ends, but by tangentially lying threads forming part of the weft. The outer portion of this represents the "universal veil," or blematogen layer.

9. The later stages of development are in general as in *Ag. arvensis*, but the partial veil, while presenting the same general duplex character, is not so strongly developed.

10. In all three species of *Agaricus*, which I have studied, there is an external zone of fundamental tissue. The outer portion of this, not well defined, does not pass over into, or give place to, the cuticle proper of the pileus, nor the partial veil. This not well defined outer zone of fundamental tissue represents the "universal veil," or blematogen. An outer portion of the "universal veil" usually becomes torn free because of a lagging or cessation of growth. It may form delicate scales on the pileus, or disappear earlier. An inner portion of the universal veil, variable in amount, remains concrete with the cuticle of the pileus. A small portion in a very young stage lies external to the partial veil and is connected with its outer surface in the young stage. But the great increase in the partial veil, by additions from the margin of the pileus and by growth of the portion next the stem, indicate that the "universal veil" probably plays an insignificant part in the formation of the partial veil, which is a distinct structure.

11. The "universal veil" as interpreted here, is homologous with the undifferentiated "universal veil" in *Amanitopsis vaginata*. Since this zone gives rise, in a later stage of development, to the mature universal veil, or volva, in the *Amanitae*, it may be called a *blematogen layer*, or *blematogen*.

12. In *Agaricus campestris*, under certain conditions, particularly those of culture, there is often manifest an additional universal veil of delicate floccose character, easily separated from the young carpophore, which in age is found as small white floccose patches on the surface of the pileus. This may be called the primary universal veil, or, to be more exact, the *protoblema* or *protoblem*. When present it is external to the *blematogen layer* which, in the species of *Agaricus* studied here, is never differentiated into a distinct universal veil, or *volva*, = the *teleoblema*.

DESCRIPTION OF PLATES I AND II

The photomicrographs were made as follows: Figs. 1–6 with an extension camera and Zeiss lenses, × 15 diameters. Figs. 8–11 were made with a Zeiss microscope, the object being 370 mm. from the sensitive plate; figs. 7, 10 and 16 with ocular no. 4 and objective no. 16 mm.; fig. 8 with ocular no. 8 and objective no. 3 mm.; fig. 9 with ocular no. 12 and objective no. 16 mm.

FIG. 1. Longitudinal section of very young carpophore of *Ag. arvensis* showing earliest origin of gill slits as two symmetrically disposed light spots, separating pileus fundament above from the stem fundament below, indicating a constriction between them. External to the fundament of the pileus and stem is the fundament of the "universal veil," or the blematogen layer. It is easily recognized in this figure by the more open mesh of its tissue compared with the denser tissue of the pileus and stem fundaments, and stains darker because the thick walls of the hyphae take up the stain readily. The base of the young carpophore is lighter colored than the stem fundament indicating that growth is more active in the latter. The rhizomorph is attached to the base.

FIG. 2. Same in a little older stage, the gill slits are evident, the hymenophore primordium is well organized as also the primordium of the pileus margin shown by the deeper stain over the gill slits. Note the oblique position of the gill slits rising outward and upward, also shown in fig. 3.

FIG. 3. Same in a still more advanced stage. The pileus margin is more definite and the inner limit of the blematogen layer is more distinct. The outline of the stem is more distinct showing its present form to be shorter than broad. In figs. 2 and 3 there is shown the exfoliation of a very thin layer from the carpophore. This may represent the primary universal veil, or protoblem, present sometimes on young carpophores of *Ag. campestris* in addition to the blematogen, or it may represent merely a dead outer layer of the blematogen which was in contact with the substratum; it is difficult to determine this point on carpophores developed in the substratum. A similar exfoliating layer is shown in figs. 6 and 10.

FIG. 4 is a section of a young carpophore of *Agaricus arvensis* or a closely related species, collected in the edge of the forests south of Pontarlier in 1905. If it is not *Ag. arvensis* it is probably *Ag. flavescens* Gillet, as young carpophores of this species were collected, but the number became detached. It differs from fig. 1 chiefly in the very deep stain of the hymenophore primordium, and shows also a dome-shaped primordium of the pileus connecting with the primordium of the hymenophore and pileus margin, though not so deeply stained. The blematogen layer is very deeply stained due to the absorption of the stain by the thickened hypha walls. The section was not decolorized to the extent of that of fig. 1, but if it were the primordium of the hymenophore would stand out strongly as compared to that in fig. 1. The reactions here are more like those in specimens of *Ag. campestris* studied.

FIG. 5. Sections of an older stage of *Ag. arvensis* than shown in fig. 3. The position of the gill "slit" is now reversed, sloping downward. The fundaments of the lamellae are beginning to show as low folds. The outline of the surface of the stem is very distinct as a downward and outward sloping dark area below the partial veil. The surface of the primordial pileus is nearly organized, its elements interlacing with the inner layer of the "universal veil," or blematogen which still shows the coarser mesh. The partial, or marginal veil shows a section of the blematogen or

"universal veil," as its outer surface, but the bulk of it is formed by the growth of threads from the margin of the primordial pileus and increase of its own elements. The duplex character is beginning to show, the lower portion showing a more open mesh, increase having come chiefly from growth of fundamental tissue between the blematogen and stem surface.

FIG. 6. Section of a somewhat older carpophore of *Ag. arvensis*, the gill cavity slopes downward still more due to continued epinasty of the pileus margin and the elongation of the stem; the duplex character of the veil is more distinct; the bulb of the carpophore has broadened greatly but has not elongated appreciably so that the stem surface here is horizontal while the main part of the stem is elongating, which brings the surface nearer a perpendicular position. The open mesh character of the medulla is beginning to show due to a lagging behind in growth. The primordial surface of the pileus has become concrete with the inner zone of the blematogen, or "universal veil," so that its outer zone really becomes the surface of the mature pileus.

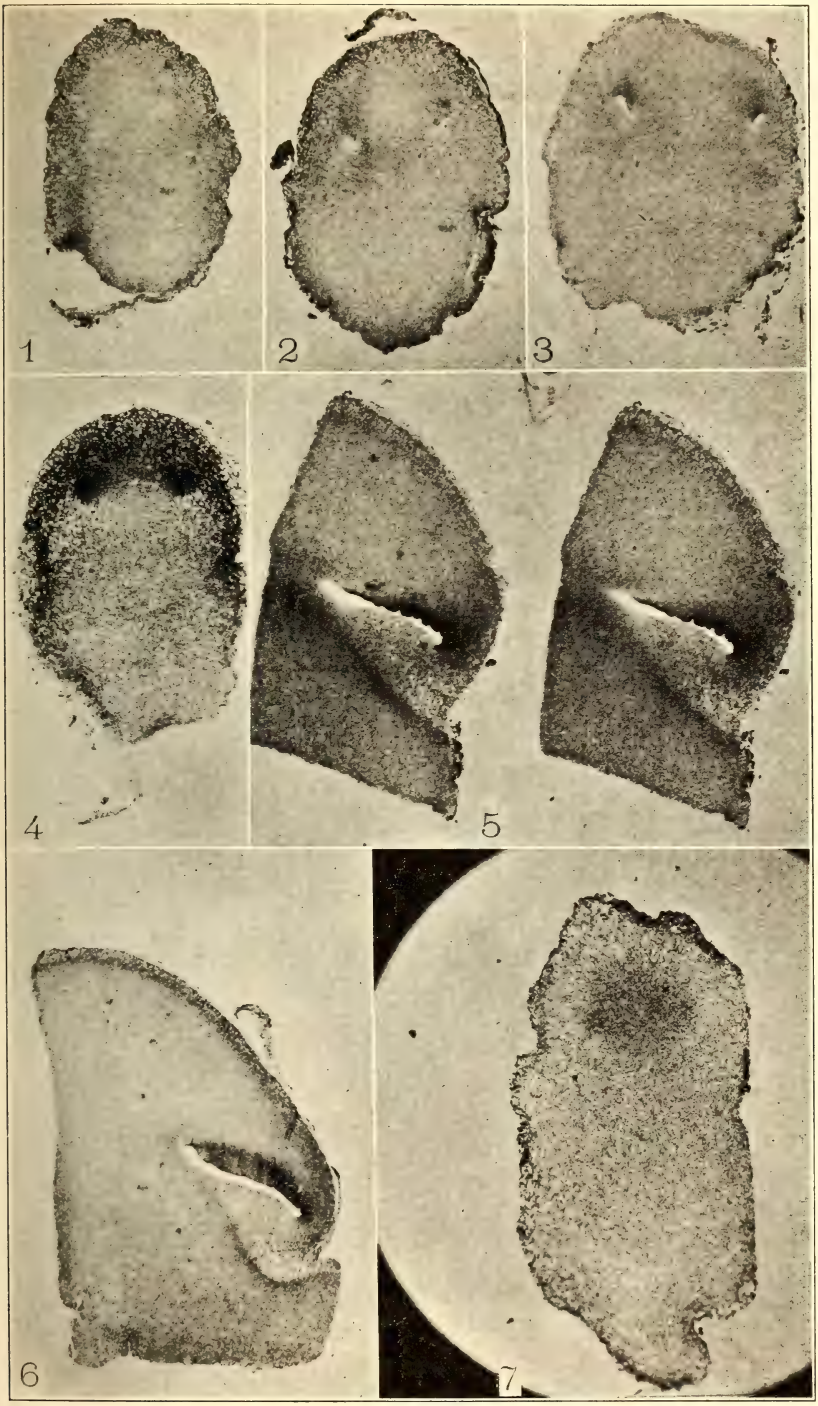
FIG. 7. Section of a young carpophore of *Ag. comtulus* showing in the upper portion the roundish primordial area of the pileus, on either side the more densely staining primordium of the hymenophore and pileus margin; below the nascent primordium of the stem, enveloping stem and pileus fundaments, is the coarse-meshed blematogen, or "universal veil."

FIG. 8. *Ag. arvensis*, highly magnified portion of fig. 1 showing details of structure and differentiation in the region of the early primordium of the hymenophore and pileus margin. This is located at the intersection of lines perpendicular to *a*, *a*. At the right note the coarse-meshed tissue of the blematogen with its thick-walled hyphae, in strong contrast with the dense area at the left with thin-walled hyphae. At the angle of this tissue (intersection of lines from *a*, *a*) note curving downward of the elements of this primordium. The open-meshed tissue beneath is the beginning of the gill cavity, and the threads of this tissue form the primordium of the inner portion of the partial veil; the hyphae are thin-walled and distinct from those of the blematogen lying outside.

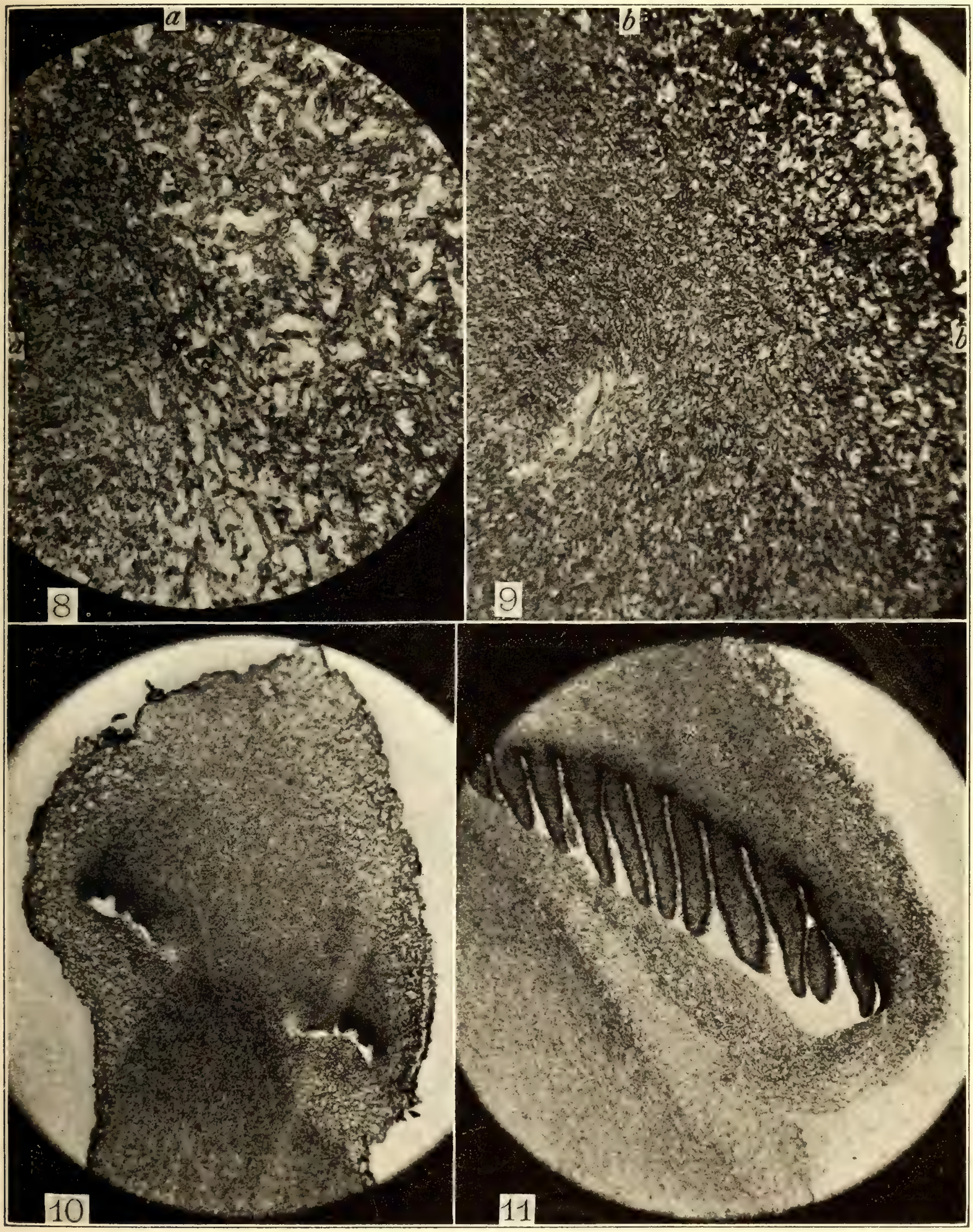
FIG. 9. *Ag. arvensis*. Highly magnified portion of a section from the same carpophore as fig. 2, showing young gill "slit," the hymenophore primordium just above; the primordium of the pileus margin above and slightly to the right, at intersection of perpendicular lines from *b*, *b;* on the right the open-meshed tissue of the blematogen, or "universal veil," below the margin of the pileus and the gill "slit" is the now more abundant tissue of the partial veil of finer texture than that of the blematogen.

FIG. 10. *Ag. comtulus*. Section of well advanced carpophore, showing the hymenophore primordium with nascent lamellae; the distinct primordial margin of the pileus, the less differentiated area of the pileus primordium above; the loose meshed blematogen, or "universal veil," the well advanced partial veil of duplex structure below the gill slit covered externally by a section of the blematogen; the conical primordium of the stem below.

FIG. 11. *Ag. comtulus*. Section of a nearly mature carpophore, slightly tangential, showing nearly mature lamellae; duplex partial veil; surface of stem; and pileus surface "concrete" with the "universal veil," or blematogen; a section of the latter forms one-third to one-half the thickness of the portion of the partial veil extending from margin of pileus to its junction with the lower portion.



ATKINSON: DEVELOPMENT AGARICUS ARVENSIS AND A. COMTULUS.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ATKINSON: DEVELOPMENT AGARICUS ARVENSIS AND A. COMTULUS.

STUDIES OF TERATOLOGICAL PHENOMENA IN THEIR RELATION TO EVOLUTION AND THE PROBLEMS OF HEREDITY

I. A STUDY OF CERTAIN FLORAL ABNORMALITIES IN NICOTIANA AND THEIR BEARING ON THEORIES OF DOMINANCE[1]

ORLAND E. WHITE

When Mendel's law was rediscovered, dominance was considered as essential and as important a principle as segregation. Further investigation soon demonstrated the phenomenon of "imperfect dominance," and still later studies led to a substitution of the "presence and absence" factor hypothesis for Mendel's conception of contrasted character pairs. De Vries (1902), Bateson (1909), Davenport (1910), Castle and others look upon dominance as an attribute of the factor or determiner, and according to the last two investigators, variation in dominance, at least in part, is the result of variable potency, or variation in the power of a determiner or factor to express itself in ontogeny. De Vries held the racially older characters to be dominant over the younger, a conception which the last ten years of experimental investigation has not upheld. On the other hand, East (1912) and Emerson (1912) think of dominance as a result of the activities of one or more specific factors, plus the modifications produced by the whole factorial organic complex (all the other factors concerned in the organism's heredity) and by the external environment (climate, soil, etc.). In other words, under identical genotypical and external environments, the factor A would always give the same expression, no matter how often the experiment was repeated.

The chief value of the data which I have to present lies in its bearing on this important question of dominance. The abnormalities concerned are three in number, viz., petalody and pistillody of stamens and that peculiar form of corolla doubling to which de Vries and others apply the term catacorolla. The data on each are given in some detail, followed by a short discussion and summary.

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[1] Contribution from the Laboratory of Genetics, Bussey Institution of Harvard University. Brooklyn Botanic Garden Contributions, No. 7. Read at the Annual Meeting of the Botanical Society of America, Atlanta, Ga., 1913.

The work was done in the Laboratory of Genetics, Bussey Institution of Harvard University, under the direction of Prof. E. M. East, for whose kindly interest and criticism, I wish to express my appreciation.

The material was obtained from various pure line cultures of *Nicotiana* species, which had been under observation for several years. All pure species used in this study bred comparatively true and no abnormal variations appeared in them, except in *Nicotiana langsdorffii grandiflora*, which was subject to petalody, and gave evidence of being a hybrid as it was heterozygous for yellow and blue pollen, the true form according to Comes (1899) having only blue pollen.

1. Petalody

This teratological character is an extremely common feature of garden flowers, and, as usually found, is variable even among the stamens of the same flower, *i. e.*, one stamen may possess it, or it may be present in two, three, four or all of them. On one stamen, the petal-like outgrowth from the filament, which constitutes the character, may be very small, while another filament in the same flower may show an anomalous enlargement from three to ten or twelve times as great. It presents its extreme form in the common double-flowered races of *Dianthus*, *Rosa*, *Prunus* and *Ranunculus*. The majority of gardeners as well as many scientists believe that such double-flowered races can be *created* from single-flowered varieties by selection. A very excellent treatment and historical résumé of this subject is given by de Vries (1906, Chap. 17) in which he produces historical proof that many of our common double-flowered races arose suddenly and in full possession of their peculiar character. His experimental studies led him to assign doubleness because of its variability, to the category of "ever sporting" characters. In many of our cultivated races, double-flowered plants quite faithfully reproduce themselves if they are fertile at all. The majority of these races have arisen as mutations, the causal factors of which are largely unknown. Among horticulturists the belief is prevalent that intense cultivation is responsible for the anomaly, but there are no data from controlled experiments to support such a belief. Peyritsch (Goebel 1900, I, p. 195) induced all degrees of doubling in the floral organs of

Cruciferae by artificial parasitization with *Phytoptus*, and, according to Hus (1908), Molliard caused the formation of double flowers by mechanical irritation. From these facts, one may conclude that double flowers may result from many different causes.

In *Nicotiana*, petalody arose in at least two dozen plants of four or five hybrid families on which observations were being made for other purposes. The pure species from which these hyrbids were derived, while under observation for five years, never developed petalody. Further, this abnormal condition was never observed in F_1 hybrid generations, although thousands of flowers were examined.

Two of these abnormal plants were self-fertilized, and the progeny, grown under approximately the same environment as the mother plant, reproduced the character, showing it to be a hereditary and not an induced phenomenon. One of the races was derived from an F_2 segregate of *N. langsdorffii* × *N. forgetiana*. The expression of the character in the stamens was very variable. Table 1 gives a general idea of the extent of this variability among the different flowers of the mother plant. The progeny, over 100 in number, all possessed the abnormality. The throats of the corolla tubes in some plants were, however, almost packed with anomalous stamens; while in others, perhaps only a single stamen was malformed. An examination of the progeny plant by plant for differentiating characters showed that segregation in flower color, habit of plant, leaf shape, etc., had occurred, indicating that the mother plant was heterozygous for a large number of factors.

TABLE 1

Number of affected stamens per flower	1	2	3	4	5	Total
Number of flowers	1	14	4	6		25

The other race of these anomalous stamen-bearing plants was derived from selfed seed of a plant which appeared to be *N. langsdorffii grandiflora*. The variability of the abnormal character is shown in Table 2. In 1912 under the same field conditions, 70 plants were grown from selfed seed of this mother plant. The inspection of these 70 plants showed the parent to have been homozygous in all its

TABLE 2

Affected stamens per flower	1	2	3	4	5	Total
Number of flowers			1	20	4	25

grosser morphological features, excepting pollen color. Habit, foliage, height and floral characters were in all plants practically of the same type and no evidences of a difference in genotypical constitution were to be observed except for the case mentioned. The anomaly expressed itself to about the same degree in all 70 plants, and had I desired to begin selection work toward securing a double-flowering *Nicotiana*, one plant would have been as good a starting point as another.

Summarizing these facts, one finds that where the anomalous race was heterozygous in many characters, the expression of petalody was extremely variable; while in the race largely homozygous, practically no variation in the abnormality was noted.

2. Pistillody

This anomaly consists of the presence of small pistils in connection with the anthers. Sometimes these little pistils amount to no more than a style and a stigma; at other times, the anther or pollen-sacs may be partly changed into carpels and rudimentary ovules produced. Occasionally such ovules are fertile and produce seeds. An examination of the literature on the subject shows the character to be neither common nor rare. Usually it is so small and inconspicuous that it passes unnoticed, but in the opium poppy, it is showy and characterizes a distinct horticultural variety. *Papaver somniferum* var. *monstruosum* or var. *polycephalum*, as it is sometimes called, affords the material for a very interesting chapter on pistillody in "Species and Varieties, their Origin by Mutation" (de Vries, 1906, Chap. 13). The writings of Masters, DeCandolle, and Hofmeister also contain valuable information on this subject. Masters considered the anomaly to be an accidental phenomenon, while DeCandolle in his Prodromus described pistilloid wall flowers as a distinct variety. The pistilloid poppy is at least a century old, and was grown as a field crop in Europe, being especially valuable because its anomalous condition did not allow the capsule to open and scatter the seed. De Vries (1906, pp. 369–99) found these poppies, in respect to their chief peculiarity, very sensitive to environment, especially during the first two to five weeks of their seedling stages. By manipulating the soil conditions at the proper time, he was able to increase and decrease the anomalous expression. Plants almost normal and those extremely abnormal were produced in this manner. Selection had no permanent effect on its expression. De Vries classified it as an "eversporting" variety. Although it was possible almost to destroy the character or inhibit the expression of its hereditary elements by modifying the environment, it was never absolutely eliminated by this treatment. In addition to the action of the external surroundings, internal factors must have had some part in making this an extremely sensitive character, because poppies, like corn, are cross-fertilized, and hence are more or less heterozygous, and, while the external conditions are no doubt very important for the characteristic development of the anomaly, the eversporting condition one may ascribe at least partially to the effect of segregating genes.

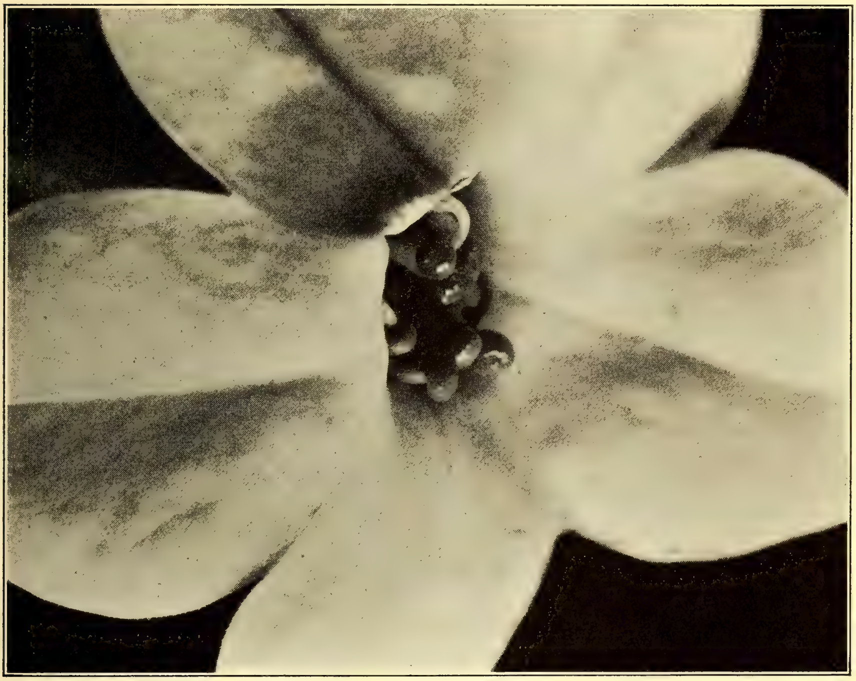

FIG. 1. *Nicotiana* flower showing pistillody.

The race of pistilloid *Nicotianas* with which I experimented originated from the guarded seed of a single anomalous mutant which was discovered among the segregates of an F_2 generation from *N. langsdorffii* × *N. alata*. Two or three hundred of these F_2 plants from the same cross were grown, but no other pistilloid mutant was found among them. The plant was designated (-2-1A) and in all subsequent experiments will be known under this number. Over 110 of its flowers were examined, all of which showed the character in each stamen, although there was considerable quantitative variability. No semblance of an ovary in connection with the pistilloid stamens was found in these -2-1A flowers, although this occurred in its descendants. Cuttings of the mutant were made, and selfed seed procured from which 90 offspring were obtained, 72 of which reproduced the character in all faithfulness, and were in all apparent respects like the parent. Eleven of the progeny developed flowers with only two or three or at most four pistilloid stamens, and in these, the anomalous pistils were much smaller than those of the original (-2-1A) or of its 72 offspring. Seven of these offspring entirely lacked pistilloid stamens. At first, such a state of affairs was very puzzling, as the possibility of technical error was not taken into consideration. However, there were sap-colored flowers among the progeny, which was

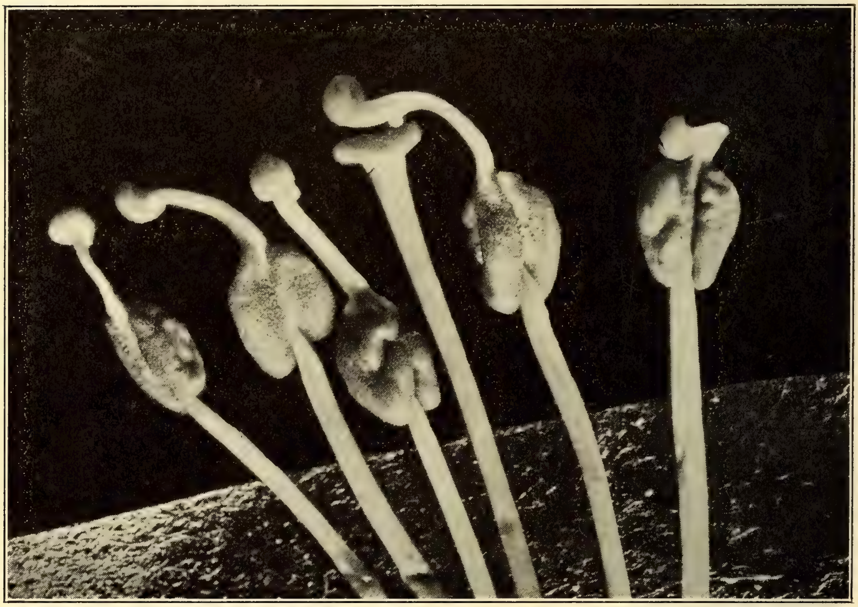

FIG. 2. Stamens from a single flower showing pistillody in detail.

very surprising, inasmuch as the hybrid family had contained only cream and white-flowered plants even to the grandparental generation. Table 3 shows the ratio of white to colored plants and their stamen character.

TABLE 3

Color	Pistillody	Pistillody not fully expressed	Normal	Total
White	71	10		81
Colored	1	1	7	9
	72	11	7	90

When I found that some of the progeny with sap-colored (magenta, etc.) flowers possessed pistilloid stamens, I was more puzzled than ever, because I had already found it to be completely recessive in the crosses I had made. When the conception of dominance and recessiveness as characteristics, not of the unit "character" or factor alone, but of the latter plus the effect produced upon it by its internal (genotypical) and external environments, was brought to bear upon the problem, the explanation was simple, especially as 90 F_1 and 381 F_2 progeny of a cross between -2-1A and 321 (*N. alata*) had given nothing but white-flowered plants. During the winter I had been working with many colored-flowered F_2 segregates of *N. forgetiana* (314) × *N. alata* (321) and had not been careful enough about cleaning my pollenizing tools before selfing the flowers on the cuttings of the original (2-1A) mutant, and, as a result, a few hybrid seeds were produced. Pistilloid stamens in the colored-flowered plants were due to dominance, complete in one case and partial in the others, of the anomalous condition over that of the normal. In the other 7 progeny with colored flowers, the expected condition, *i. e.*, the dominance of the normal, prevailed. Probably all 18 progeny belonging to the normal and intermediate classes were hybrid. Further experiments are in progress to determine this. The change in dominance is not thought to have any special connection with the color factors, but is interpreted in the same manner as the anomalous results secured in some of my unpublished studies on fasciation, viz.: the modifying influence of other factors. The 18 plants which were causing confusion had, in the majority of cases, a very different and distinct habit from the original pistilloid mutant, and this was especially true of the plants with colored flowers. The 72 or more pure abnormal (2-1A) progeny were very similar in habit, flower color and other characters, so much so that I inferred that the parent plant (2-1A) had been largely homozygous in its genotypical constitution.

From the cross referred to above (2-1A × 321), 90 F_1 progeny were grown, all of which were intermediate in both habit and in size of floral organs, but *absolutely* normal as regards pistillody. Two of these were selfed and F_2 progeny grown. The results are tabulated in Table 4.

TABLE 4

Pedigree	Normal	Abnormal	Total
(394 × 321) — 2 — 1A × 321 — 1	103	82	185
(394 × 321) — 2 — 1A × 321 — 2	152	42	196
Total	257	124	381
Expected	285.75	95.25	381
Deviation	—28.75	+28.75	

One family (-2) gave a fair approximation to the 3:1 ratio, but the other had a large excess of abnormal segregates, which I am at present unable to account for, because the two families were grown from the same grandparental stock, and under the same external environment. Many other characters of a structural nature had segregated in this F_2 generation, and the variation in the expression of the anomaly was large. Many plants were as abnormal, and many much less so than the grandmother. Other abnormalities appeared, both in pistilloid and normal segregates. Split corolla tubes and 3- to 4-loculed ovaries were not infrequent. Some of the segregates, as well as a number of the pure line (?) progeny, possessed flowers with pistilloid anthers containing numerous small ovules. Where these occurred, the pollen-sacs were deformed, sterile, and usually the ovules were exposed, owing to hypertrophy of the anther-sac walls.

3. CATACOROLLA

This is not an uncommon anomaly, and hereditary races of it have long been known, *e. g.*, hose-in-hose primula, and a garden variety of gloxinia, first described by Prof. E. Morren (see Masters, 1869, pp. 451–52, figs. 213–14). Catacorolla has been exceedingly well described by both Morren and Masters, so I shall not take the space here for a general detailed description, but confine myself to the form it takes in the particular race with which I worked. This race (4-1A) is descended from a single plant which possessed the catacorolla peculiarity to a more marked degree than any other one of the 15 anomalous plants which appeared in a family of 50 F_2 segregates from a cross between *N. langsdorffii* × *N. alata*. In fact, this hybrid family was derived from the same grandparental cross as that in which the pistilloid mutant occurred. Instead of a bud mutation occurring shortly after fertilization, as was probably the case with the homozygous pistilloid character (-2-1A), this catacorolla mutant (-4-1A) must have originally arisen as a change in the gametes of one or the other of the grandparental types or in the cells concerned in their ontogenesis, if we are to interpret the succeeding experimentally obtained results in accordance with our general knowledge of heredity. In the F_2 generation grown from guarded F_1 seed from a cross between two normal individuals occurred a segregation of 15 anomalous and 35 normal plants, making a ratio of 7 normal to 3 abnormal or 2.33 : 1. Supposedly the abnormals would have all bred true, for the one plant (-4-1A) which was selfed produced 20 progeny all of which faithfully repeated the parental peculiarities in respect to catacorolla, habits of growth, character of foliage, size and color of flowers, and color of pollen. It is not supposed that only one "altered" egg cell or pollen grain was necessarily produced in attempting to explain the place in ontogeny at which this mutation arose. Possibly many were formed as the result of a prematuration mutation, but if such were the case, and if they united with unaltered gametes, the resulting seeds possibly were not planted, or if planted, only one F_1 plant of this sort chanced to be included in those selfed for further propagative purposes.

Catacorolla in this race is typical of the anomaly as it appears in other plant species. Petalloid segments are produced outside the ordinary corolla, and partially adhere to it, these segments having colored outer and plain green inner surfaces. In other words, the normal corolla appears to have been separated at some time during its ontogeny into five segments. Later when these fused to produce the normal gamopetalous *Nicotiana* flower, the union appears not to have taken place through the careful growing together of the edges of each segment, but on the contrary, to have been brought about in such a manner as to leave a seam like that made by a tailor. At the point of union of two segments, there is a slight waste of material, and it is this which is reflexed back in the mature flower and gives the catacorolla effect. The segment then is really a piece of left-over petal. In some flowers, the petaloid segments are not united with the normal corolla except at their bases, and, in such cases, other factors have interfered and effected a distinct separation. The anomalous character, then, is the result of imperfect fusion of the corolla segments in ontogeny. This theory is further supported by the relation that exists between the number of normal corolla lobes and the number of extra-corolla segments. Table 5 shows the character of this relationship in 28 flowers taken from the original parent (-4-1A).

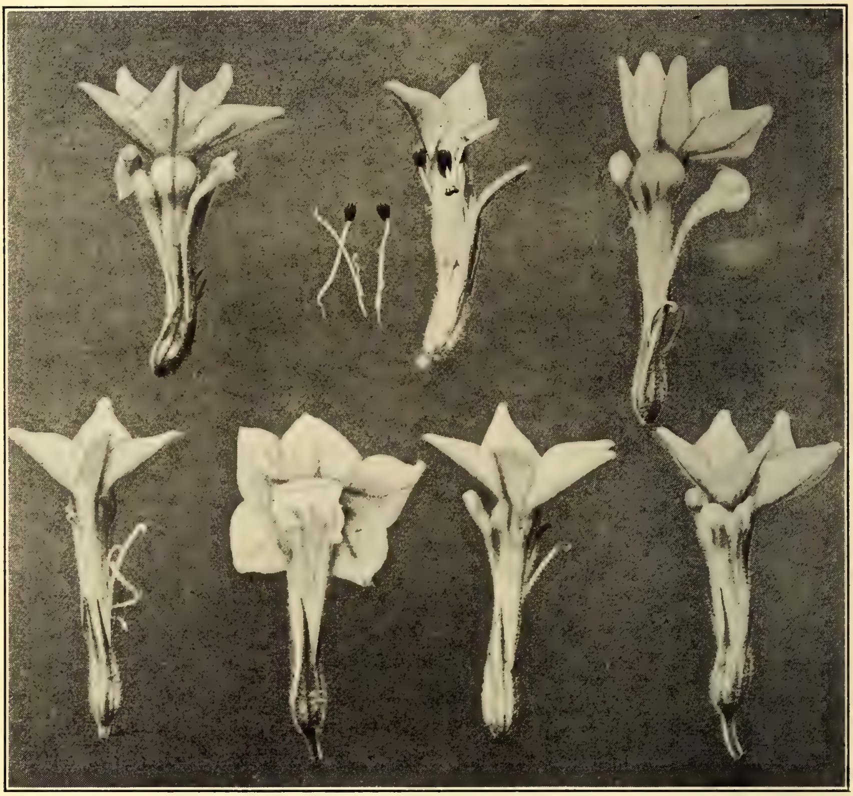

FIG. 3. "Catacorolla." *Nicotiana* flowers from the parent plant of the -4-1A race, showing variation in the expression of the anomaly.

TABLE 5

Number of segments per flower	1	2	3	4	5	6
Number of flowers	3	2	6	9	5	3

A more extended investigation from the standpoint of anatomy and morphology is necessary before such a theory can be demonstrated as a truth. The fact that six extra-corolla segments are sometimes present can be explained by supposing that two segments sometimes result from a single "seam." The size of these segments varies from a slender, thread-like structure to one as broad as the normal lobe. In some flowers they fuse and produce a supernumerary corolla. This variability is characteristic of the race as a whole, *i. e.*, some plants are not more variable than others, so that the character may be said to be eversporting only in the sense that a single plant may possess both very abnormal and slightly abnormal flowers.

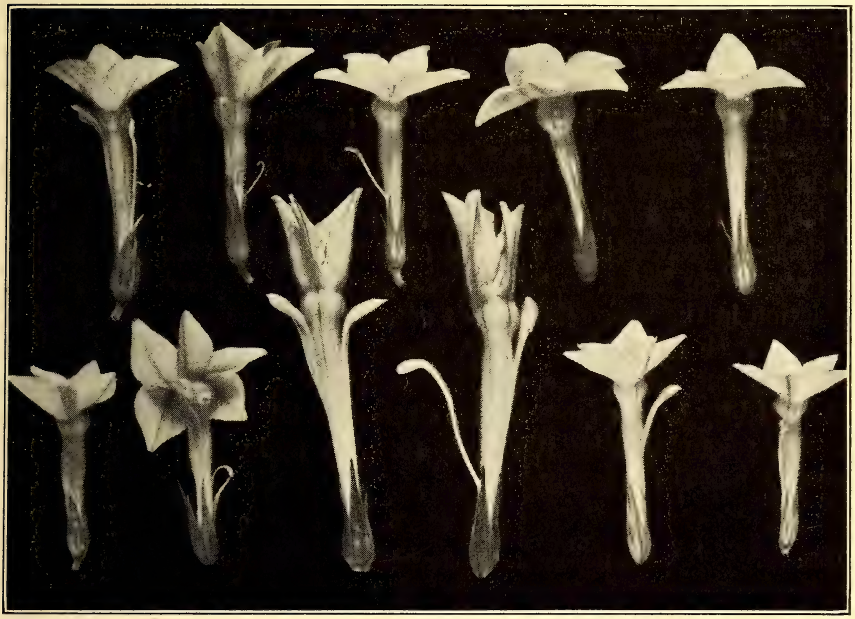

FIG. 4. Flowers from F_1 hybrids between catacorolla and normal races, showing variation in expression ("dominance and recessiveness"). Each flower represents the typical expression in a single hybrid plant.

Several series of hybridization experiments are in progress, but they have reached only the F_1 stage. The most interesting of these experiments relates to a study of the dominance and recessiveness of catacorolla. In addition to the selfed seed produced by the parent

(-4-1A) plant, a large amount of "open field" seed was gathered from it. Thousands of hybrid F_2 segregates of various crosses such as *N. forgetiana* × *N. alata* and *N. alata* × *N. langsdorffii* were grown in the same field, and in the same year as the -4-1A parent. These were all normal in respect to catacorolla, excepting the 15 plants already mentioned. Cross-fertilization was more favorable to the production of seed on this one (-4-1A) selection than self-fertilization. This means that the open field seed would produce largely hybrid F_1 plants. One hundred and sixty-two plants grown from this seed gave 43 homozygous 4-1A progeny and 119 hybrid F_1 progeny, the latter representing almost as many different F_1 combinations as there were individuals. As a consequence, they were extremely variable in almost every taxonomic feature,—in habit, height, foliage; in flower color, size and shape; in pollen color, and in many other less prominent characters. Sixty of the 119 were colored, and 59 were white. Some of the flowers were as small as those of *N. forgetiana*, while others were as large as those of *N. alata*. Fig. 4 is an attempt to show something of these differences in flower size, as well as in the variability of the catacorolla character. Each flower represents a single plant. The F_1 variation in the expression of the catacorolla was remarkable. Supposedly each of the 119 plants represented a different genotypical complex, and hence one would, on the conception of dominance supported by East, expect a great deal of variability. Table 6 shows the results of classifying the whole 162 progeny by color and by their expression of the anomaly.

TABLE 6

Color	-4-1A Pure Homozygote	Hybrids Intermediate	Hybrids Normal	Total
White	43	33	26	102
Colored		11	49	60
	43	44	75	162

Those classified as normals showed absolutely no expression of the character.

Guarded crosses were made between the -4-1A and -2-1A strains. The genotypical constitutions were very difficult, as each had a distinct growth habit, leaf size, etc. About 150 F_1 plants were grown in the same field and under approximately the same conditions as the other "catacorolla" cultures. In this cross, the F_1 expression of catacorolla was intermediate, with a fluctuation towards complete dominance of the normal, although never approaching that state. The pistillody was absolutely recessive.

4. Discussion and Summary

1. *Nicotiana* plants showing petalody were selfed and progeny grown from them. In one race the abnormal character was extremely variable, some plants showing a large expression, other plants showing it only to a slight degree. This race varied in many other characters, proving the mother plant to have been very heterozygous. In another race, the abnormality was reproduced in all the progeny to the same degree as in the mother plant. With the exception of pollen color, no variation in other characters occurred in this race.

2. Pistillody originated as a discontinuous variation and was inherited in the same manner, crosses with the normal in one case giving in F_2 a progeny closely approximating a simple 3 : 1 ratio. In two hybrid F_1 families, it was completely recessive, while in what appears to be another hybrid F_1 family, it is wholly dominant. The first two families differ from the last family in a large number of characters, as the ancestry of the latter involves another species.

3. The catacorolla race of *Nicotiana* originated from a discontinuous variation. When crossed with normal races, the F_1 progeny were either intermediate in character or absolutely normal, though the individual F_1 progeny from each cross showed no variation among themselves. Great variation existed between the different pollen parents of many of these F_1 individuals.

As a whole, the data secured from hybridizing races of normal plants with those possessing the three abnormalities discussed above support the view that dominance and recessiveness are not in any way attributes of the factor or "character" in itself, but are the result of the factor expression plus the modifying influence of the environment, whether genotypical or external (soil, climate, etc.). The variability in the expression of catacorolla in the 119 F_1 plants of -4-1A crossed with the 119 different normals is strong supporting evidence that this conception of dominance is the most tenable of those recently advanced by geneticists.

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NUCLEAR BEHAVIOR IN THE PROMYCELIA OF CAEOMA NITENS BURRILL AND PUCCINIA PECKIANA HOWE[1]

Louis Otto Kunkel

In a preliminary paper (10), I have shown that the aecidiospores of *Caeoma nitens* Burrill produce promyeclia. The behavior of the nuclei during the germination of these spores was not described at that time and has been the subject of my further studies.

Tulasne (19) in 1854 found that the aecidiospores of *Endophyllum Euphorbiae silvaticae* do not germinate as normal aecidiospores. Instead of forming non-septate germ tubes, these spores produce promycelia. Morphologically considered they are aecidiospores; nevertheless, they function as teleutospores. Sappin-Trouffy (17) also studied them and confirmed the observations of Tulasne as to the manner of their germination. According to Sappin-Trouffy, however, the behavior of the nuclei in the germination of this aecidiospore differs from that in a normal teleutospore. The two nuclei in this case do not fuse but pass directly out into the tube of the promycelium where they divide to produce the four nuclei.

Maire (12) also made a cytological study of the aecidiospores of *E. Euphorbiae silvaticae.* He agrees with Sappin-Trouffy that nuclear fusions do not occur in them. He also studied the aecidiospores of *E. sempervivi* and decided that here again the nuclei do not fuse but pass out into the germ tube and divide, just as in the case of *E. Euphorbiae silvaticae.*

More recently Hoffmann (8) has made a very careful cytological study of *E. sempervivi.* He finds that a nuclear fusion does occur in the aecidiospores of this rust and that the four nuclei of the promycelium arise by two successive divisions of the fusion nucleus, just as in the case of ordinary teleutospores. He showed that the first or even both of these divisions may take place in the aecidiospore. This observation suggests that the two nuclei, which according to Maire (12) pass out of the spore into the promycelial tube are the product of the first division of a fusion nucleus. In the light of Hoffmann's work it seems highly probable that nuclear fusions also occur in the aecidiospores of *E. Euphorbiae silvaticae*. Sappin-Trouffy (17) and Maire (12) were probably both wrong in supposing that the nuclear phenomena connected with the production of promycelia by the aecidiospores of *Endophyllum* are different from those to be observed in the case of teleutospores.

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[1] Contributions from the Department of Botany, Columbia University. No. 259.

Although the production of promycelia by the aecidiospores of *E. Euphorbiae silvaticae* and *E. sempervivi* is well established, certain observations have been made which have been regarded as indicating that these aecidiospores may at times germinate in the ordinary fashion. Thus Nypels (15), Maire (12), and Werth (20) claim that occasionally they find non-septate germ tubes which are like the germ tubes produced by the aecidiospores of other rusts. Werth noted that the aecidiospores of *E. sempervivi* produce non-septate germ tubes when immersed in water during germination, but that if floated on the surface of the water, they produce typical promycelia. He seems inclined to think that these non-septate germ tubes can infect the host plant. Hoffmann (8), however, has failed to find any evidence that the aecidiospores of *E. sempervivi* ever produce the ordinary type of germ tubes. It is evident that a further cytological study of these aecidiospores is needed to settle this important question. If it could be shown that nuclear fusions occur in them and that their tubes typically contain four nuclei, which are the product of two successive divisions of this fusion nucleus, then, even in the absence of septa, the promycelial nature of the tubes would be established. If on the other hand it could be shown that nuclear fusions do not occur under the conditions mentioned, but that the two nuclei pass out into the germ tube and there divide conjugately, we should be compelled to admit that the type of germination is dependent on environmental conditions. In the absence of direct cytological evidence, however, we must be very slow to accept such claims.

My observation that the aecidiospores of *Caeoma nitens* Burrill also produce promycelia, indicates that this rust has a life history similar to that of the two species of *Endophyllum* noted above. *Caeoma nitens* is very widely distributed and produces aecidiospores in great abundance. It thus furnishes excellent material for the study of the questions that have been raised in connection with the genus *Endophyllum*. I have described the earlier work on *C. nitens* in my preliminary paper (10). Galloway (14) studied the germination of the aecidiospores in water and noted that the germ tubes which they produce may become somewhat septate, but he did not observe the production of sporidia and his drawings scarcely suggest a resemblance to promycelia. In more than twenty figures Clinton (5) shows the various stages of germination which he studied. He also notes an occasional septum in some of the tubes, but he did not obtain the production of sporidia. Tranzschel (18) and Clinton (6) both claim to have established by infection experiments that *C. nitens* is the aecidial stage of *Puccinia Peckiana* Howe. Tranzschel's experiments, however, were not guarded by checks. The data given below show that such a connection is very improbable. The work of these authors has, nevertheless, been accepted and Arthur (1) has made *C. interstitiale* Schlecht. (*C. nitens* Burrill) the type species of his genus *Gymnoconia* Lagerh. This genus is described as autoecious and as including in its cycle of development, spermatia, aecidiospores and teleutospores. The only other species in the genus is *Gymnoconia Rosae-gymnocarpae* (Dietel.) Arthur. The teleutostage of this species is not known and my work suggests that a study of the germination of its aecidiospores may show it to be a short-cycled rust with a life history similar to that of *Caeoma nitens*. At any rate it is plain *C. nitens* cannot properly be included under Lagerheim's genus *Gymnoconia*.

Olive (16) and Kurssanow (11) have found that the aecidiospores of *Caeoma nitens* normally contain two nuclei. They have also shown that sexual fusions occur in the base of the caeoma, previous to the production of spores. The nuclear phenomena covering this stage in its life history are well established and agree with what has been found by Blackman (2), Christman (4) and others in a rather large number of rusts. In my preliminary paper I showed that the aecidiospores produce promycelia, but I had not then made a cytological study of the nuclear phenomena of their germination.

The material used in my further studies was obtained from well-infected blackberry leaves. The spores were dusted on the surface of tap-water in Petri dishes. In the early spring good germination could be obtained in such cultures when they were kept at room temperature (about 23° C.), but later in the season when the weather became quite warm it was necessary to place the cultures on top of an icebox in order to secure abundant germination. Most of the spores which I have used were taken from leaves of *Rubus frondosus* that were gathered in Van Cortlandt Park, New York City. I have, however, also germinated spores that were sent to me from Urbana, Illinois; Columbia, Missouri, and Madison, Wisconsin. I find that they all produce promycelia.

Spores having promycelia in various stages of development were floated from tap-water cultures on to glass slides. They were attached to the slides by means of egg albumen, fixed, hardened in alcohol and stained with the triple stain. The ripe ungerminated aecidiospores seem difficult to fix and it was necessary to make many preparations in order to secure a sufficient number of slides that show the nuclei clearly defined and well stained. This difficulty in the fixation of the ungerminated spores is probably due to the slowness with which the fixative penetrates their dry cell walls. As soon as this wall is penetrated by the promycelial tube, the contents of the spore become easy to fix and readily stain.

In agreement with the observations of Olive (16) and Kurssanow (11), I find that the normal aecidiospores are binucleate. A study of stained sections through the caeoma, however, shows that, as Olive and Kurssanow have already observed, occasionally there are rows of spores, each of which contain more than two nuclei. Such spores are larger than the binucleate spores and their size is roughly proportional to the number of nuclei which they contain, thus maintaining nucleo-cytoplasmic ratio. Kurssanow found that these spores arise through a fusion of more than two cells in the caeoma. Two of these abnormal spores, one containing three nuclei and the other containing four nuclei, are shown in figures 1 and 2. A normal binucleate spore is shown in figure 3.

A study of the binucleate spores during germination shows that they become uninucleate previous to the production of promycelia. Preparations made from cultures that were fifteen hours old show a large number of uninucleate spores. In some of these preparations more than ninety per cent. of the spores contain only one nucleus, thus demonstrating that during the early stages of germination the binucleate spores become uninucleate. This observation strongly suggests that a nuclear fusion takes place. A large number of spores have been studied with the hope that the actual stages in such fusions could be demonstrated. The nuclei are clearly defined and can frequently be observed to lie close together and flattened against each other, but the actual breaking down of the membranes and the union of the chromatin reticula are hard to demonstrate. I find no evidence of disintegrating nuclei in any of the spores.

The normal germination of the aecidiospore consists in the pushing out of a germ tube into which the protoplasmic contents of the spore passes. See figure 5. As the nucleus travels out into the germ tube it becomes elongated, figure 6. This nucleus soon divides, figure 7, producing two nuclei which may again divide immediately as shown in figure 8. The two nuclei that are the product of the first division may round up as shown in figure 9 and may even become separated by a cross wall before the second division occurs. Figure 10 shows a spore containing two nuclei and producing a germ tube. This suggests that here as in *Endophyllum sempervivi* according to Hoffmann the first nuclear division may occur in the spore.

Nuclear division, as noted, is sometimes followed immediately by cell division, figure 11, but in other cases the four nuclei of the promycelium may be present before cross walls are formed. There is some evidence that these cell divisions take place by constriction, as has been reported in other rusts (9 and 16), but I have not studied this point especially. The second nuclear division is soon followed by cell division giving normally four cells that are filled with protoplasm and contain one nucleus each, together with a stalk cell which is without a nucleus. The stalk cell is either empty or contains a very small amount of protoplasm at or near its upper end. We have here a case in which a cell is cut off which contains no nucleus and may or may not contain protoplasm. When protoplasm is present it is massed at the upper end of the cell leaving not even a vacuole and primordial utricle in the lower end. I have occasionally observed promycelia that are composed of more than five cells. Such promycelia, however, never produce more than four sporidia and when they are stained show a nucleus in only four of their cells. Brefeld (3) has observed a similar cutting off of functionless cells in the promycelia of *Tilletia Caries*, *Ustilago Maydis* and other forms.

The sterigmata arise as pointed protrusions from the nucleated cells of the promycelium. They are generally though not always produced near the distal end of the promycelial cell. After reaching a length of from fifteen to twenty microns the extreme end of the sterigma begins to enlarge. Thus the sporidium arises through the enlargement of the end of the sterigma. At first this enlargement is almost spherical, but it soon takes on the shape of the mature sporidium.

The nucleus enters the sporidium when it has reached approximately one half the size that it will be when mature. In passing through the sterigma the nucleus becomes very much elongated, as shown in figure 12. This elongated nucleus soon becomes spherical again and takes a position near the center of the sporidium. The mature sporidium contains all or almost all of the protoplasm from the promycelial cell which is left practically empty. Generally the sporidium falls from the sterigma before its nucleus begins to divide, but sometimes, as shown in figure 13, nuclear division occurs while it is still attached to the sterigma. Nuclear division may occur in the sporidium, either before or after germination. It is, however, generally binucleate at the time it germinates. It produces either a germ tube or a sterigma-like outgrowth on which is borne a secondary sporidium. Figure 14 shows a mature sporidium in which nuclear division has not yet occurred. The nucleolus is shown outside of the nucleus in this figure. This is probably the result of fixation, since the nuclei in many of the sporidia contain nucleoli. Figure 15 shows a sporidium which contains two nuclei.

It is worth noting that if the aecidiospores are immersed in water rather than floated on its surface, they produce long tubes which are often devoid of septa. Such promycelia seldom produce sporidia and might easily be mistaken for ordinary aecidiospore germ tubes. In cultures as much as two days old, these tubes regularly contain four nuclei. A study of earlier stages of germination shows that these four nuclei arise through two successive divisions of a single nucleus. This observation establishes the promycelial nature of these germ tubes also. They are therefore not to be confused with the germ tubes of ordinary aecidiospores. Werth (20) observed similar non-septate germ tubes when he immersed the aecidiospores of *Endophyllum sempervivi* in water and is inclined to the view that under these conditions the aecidiospores produce ordinary germ tubes rather than promycelia. My observations suggest that a cytological study of the aecidiospores of *E. sempervivi* which have been germinated under water will show that they produce only promycelia. Maire (12) claims to have found a variety of *E. sempervivi* (*E. sempervivi* variety *aecidioides* R. Maire) with aecidiospores that regularly produce germ tubes rather than promycelia, but it is possible that a further study may show that these germ tubes also are in reality promycelia.

In the light of the facts described, there can be no doubt that

Caeoma nitens is a short-cycled rust which has no connection with *Puccinia Peckiana*, but it has seemed desirable to make further infection experiments and to determine the nature of the mycelium that bears the teleutospores of *Puccinia Peckiana*. Clinton (5) figures the germination of these spores but he does not show the production of sporidia and from his drawings we cannot be sure that they are producing promycelia. I have, therefore, attempted to obtain further evidence along these lines.

On May 15, three blackberry plants that were badly infected with *Caeoma nitens* were removed from Van Cortlandt Park and placed in pots in a greenhouse. Nine uninfected plants were also brought in and after they had started new growth and appeared to be in a healthy condition, five of them were, on May 28, heavily inoculated with fresh mature aecidiospores of *Caeoma nitens*. They were kept under bell jars for a few days in order to secure a moist atmosphere for the germinating spores. The other four uninfected plants were used as checks. The five plants that were thus inoculated, as well as the three plants that were badly infected at the time they were placed in the greenhouse, are at present in a vigorous condition and show no signs of being infected with *Puccinia Peckiana*. I have also had under observation several hundred plants in Van Cortlandt Park that were badly infected with *Caeoma nitens* during the early spring. All of these plants were free from *Puccinia Peckiana* at the end of the growing season and I have not been able to find this rust in the vicinity of New York City.

To secure material of *Puccinia Peckiana* for study, a visit was made to a region near Old Forge, New York, in the Adirondack Mountains and to the vicinity of Glen, New Hampshire, in the White Mountains. In both of these regions, during the latter part of August, I found *Puccinia Peckiana* widely distributed and abundant on the leaves of *Rubus frondosus* Bigel. Stained sections of infected leaves, made from material obtained on this trip, show that the vegetative cells of the mycelium are binucleate, figure 16. The cells of the teleutospores in young sori contain two nuclei, but in the older sori, the cells have become uninucleate, presumably by the fusion of the two nuclei. Thus there is nothing unusual in the development of these teleutospores.

Some of the teleutospores were also germinated. They were scraped from green blackberry leaves and were spread over the surface of agar media or floated on tap-water. After an incubation period of about two days, approximately thirty per cent. of the teleutospores germinated. They germinate by producing a promycelium that normally consists of five cells, one stalk cell and four cells that bear sporidia. Both cells of the teleutospore may produce promycelia simultaneously as shown in figure 17, but more often only one cell germinates, figure 18. The mature sporidium falls off from its sterigma and under favorable conditions germinates immediately. The sporidium produces either a germ tube or a sterigma on which is borne a secondary sporidium. A germinating sporidium is shown in figure 19. In some cases, a secondary sporidium may be borne on a branch of the germ tube, as is shown in figure 20. The development and germination of these spores leaves no doubt that they are true teleutospores, and that the aecidium stage of *Puccinia Peckiana* is yet to be determined.

While collecting in the regions referred to above, I found the blackberries quite commonly infected with a rust in the uredostage. These spores correspond fairly well with the description given by Fischer (7) for the uredospores of *Phragmidium violaceum* (Schultz) Winter, and there are in the herbarium of the New York Botanical Garden, specimens collected by Prof. L. M. Underwood at Glen, N. H., and labeled *P. violaceum*. Since, however, the teleutospores of this species has not been found in this country, the determination of the uredospores must be regarded as doubtful and there is a possibility that they belong with *Puccinia Peckiana*.

The life history of *Caeoma nitens* may be outlined as follows. The sporidia belong to the gametophytic generation and produce a mycelium that is composed of uninucleate cells. Whether the germ tubes of the sporidia are able to enter various tissues of the host or can only infect the young buds of the underground stem as is believed by Müller (13) to be the case for *Endophyllum Euphorbiae silvaticae*, is a question which must be left to future studies. The uninucleate mycelium is certainly perennial in the tissues of the host (14) and produces spermagonia and aecidia on the shoots and leaves in the early spring. The aecidiospores are borne in chains and alternate with intercalary cells. Both the aecidiospores and intercalary cells are binucleate and constitute, therefore, a sporophytic generation. This binucleate condition arises through the fusion of equal cells in the base of the caeoma. With this fusion the sporophyte generation begins. In some cases more than two cells fuse and this leads to the production of aecidiospores that contain more than two nuclei. The behavior of the nuclei in these spores during germination is a problem which I have not yet been able to solve. The age of the mycelium which bears the aecidiospores is not known. It is generally supposed to be approximately one year old but this question cannot be settled without experimental evidence. It is interesting to note in this connection that Müller found that the mycelium which produces most of the aecidiospores of *Endophyllum Euphorbiae silvaticae* is two years old. The binucleate aecidiospores become uninucleate, presumptively by nuclear fusions during the early stages of germination. Two successive divisions of the nucleus of the aecidiospore produce the four nuclei of the promycelium and each nucleated cell of this promycelium produces a sporidium.

I am inclined to believe that *Caeoma nitens* is a rather primitive form among the rusts. The predominance of the gametophytic generation over the sporophytic generation is strong evidence in favor of this view. This is the condition which we generally meet with in the lower forms of plant life. Those rusts in which the sporophytic generation has become the important stage are probably higher in the scale of evolution than such forms as *Caeoma nitens* and *Endophyllum sempervivi*. *Caeoma nitens* should also be regarded as more primitive than *Endophyllum*, since it possesses a simpler form of aecidium.

I am greatly indebted to Dr. R. A. Harper and Dr. W. G. Marquette for advice and criticism while engaged in this work.

COLUMBIA UNIVERSITY,
NEW YORK CITY

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EXPLANATION OF PLATE III

All the figures were drawn with the aid of a camera lucida, and with the Leitz Obj. $\frac{1}{6}$ inch. or $\frac{1}{12}$ inch, and ocular 4.

Caeoma nitens

Magnification 1570 diameters

FIG. 1. An aecidiospore which contains three nuclei.

FIG. 2. An aecidiospore which contains four nuclei.

FIG. 3. A normal aecidiospore containing two nuclei.

FIG. 4. An aecidiospore which has become uninucleate.

FIG. 5. A uninucleate aecidiospore in an early stage of germination.

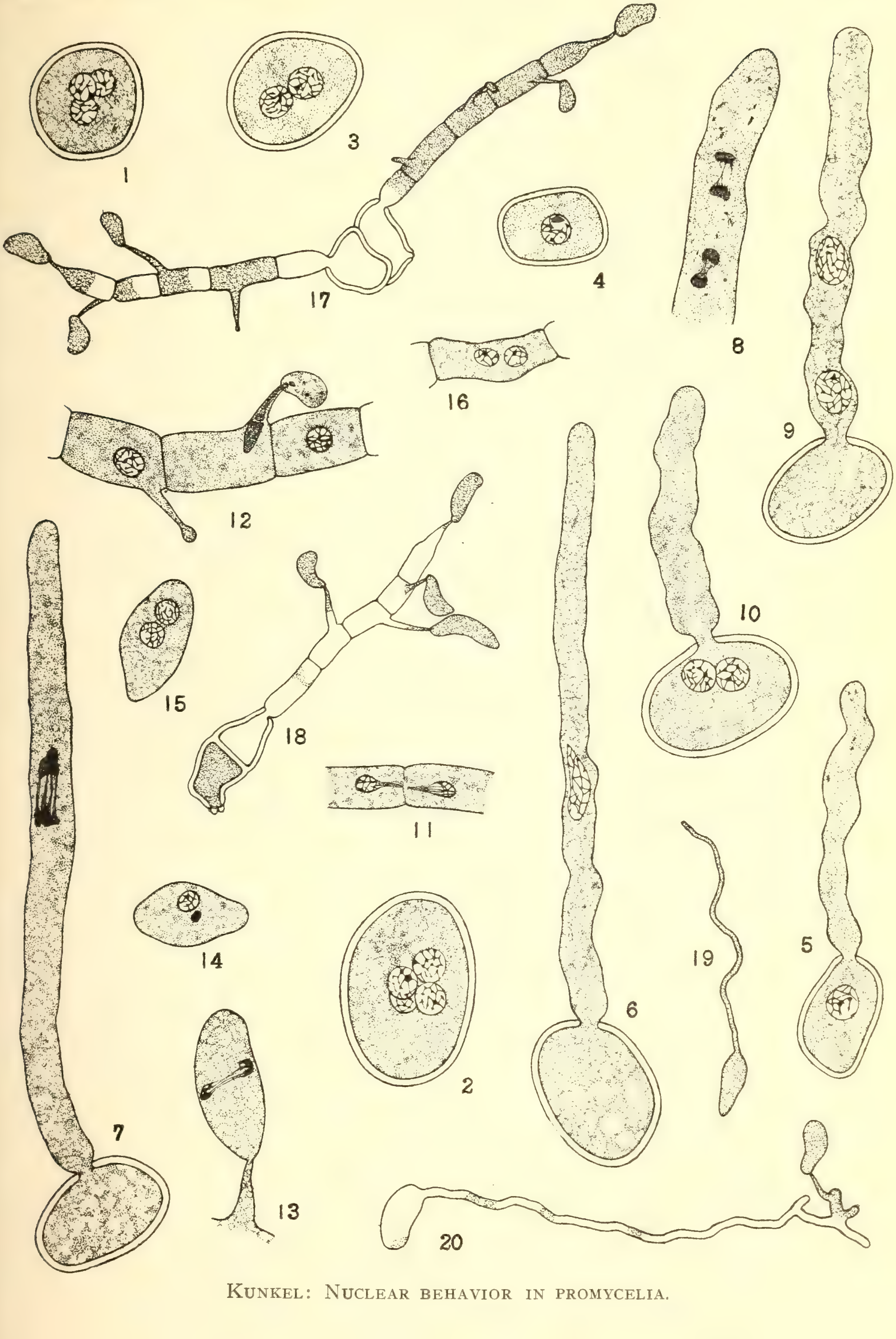
FIG. 6. A later stage of germination in which the nucleus is much elongated and is passing up the promycelial tube.

FIG. 7. First nuclear division, diaster.

FIG. 8. The second nuclear division in telophase.

FIG. 9. A promycelial tube containing two nuclei that have rounded up after the first division.

FIG. 10. An aecidiospore containing two nuclei and producing a promycelial tube. This figure suggests that the first division has taken place in the spore.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KUNKEL: NUCLEAR BEHAVIOR IN PROMYCELIA.

FIG. 11. Cell division following immediately after the second nuclear division.

FIG. 12. Passage of the elongated nucleus into the sporidium.

FIG. 13. Nuclear division taking place in a sporidium that is still attached to its sterigma.

FIG. 14. A uninucleate sporidium, showing the nucleolus outside the nucleus. This is probably a result of fixation.

FIG. 15. A binucleate sporidium, showing the two nuclei rounded up and in a resting condition.

Puccinia Peckiana

Magnification 1000 diameters

FIG. 16. A typical vegetative cell from the mycelium, showing the binucleate condition.

FIG. 17. A germinating teleutospore. Both cells of this teleutospore are producing promycelia simultaneously.

FIG. 18. A teleutospore which shows only one of its cells germinating.

FIG. 19. A sporidium which has produced a germ tube.

FIG. 20. A sporidium which has produced a germ tube on which is borne a secondary sporidium.

ON AXIAL ABSCISSION IN IMPATIENS SULTANI AS THE RESULT OF TRAUMATIC STIMULI

Ross Aiken Gortner and J. Arthur Harris

A considerable part of the very extensive literature of abscission pertains to the normal separation of the leaf from its axis, or of the leaflets of a compound leaf from their common petiole upon the approach of winter. Fifty years ago, however, von Mohl[1] beginning with the observation that the leaves of plants which are put into the press for the herbarium sometimes separate from the stems, gave a most interesting account of some of the factors involved. Since then there has been a great series of scattered observations pertaining to normal and abnormal leaf fall. Recently, we have had the detailed experimental study of the fall of the corolla by Fitting.[2] Quite dissimilar to the above studies but of some interest in the present connection are the observations which have been published here and there on the self pruning of trees.

Our purpose in this note is to call attention to abscission of the axis in *Impatiens Sultani.* This particular type of phenomenon we have never found described in the literature, but it may have been several times mentioned incidentally under titles which gave no clue to this part of their content, and which have escaped our notice because they are aside from our usual lines of work. We are fairly confident, however, that it has not been described in the *Sultani.*

Impatiens Sultani,[3] like so many other species of this genus, has very watery, translucent stems, which take root readily, either when accidentally coming in contact with the soil or when cuttings are made.[4]

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[1] Mohl, H. von. Bot. Zeit. **18**: 1–7, 9–17, 132–133, 273–277. 1860.

[2] Fitting, H. Jahrb. Wiss. Bot. **49**: 187–263. 1911.

[3] For Hooker's original description see Curtis's Bot. Mag. *pl. 6643.* For other figures see: Garden, **22**: 208–209. *pl. 352.* 1882; Illustration Horticole, **30**: 93–94. *pl. 488.* 1883.

[4] The original plants were grown from seeds accidently secured in the soil in which other plants were packed for shipment. As far as we are aware it has not produced seeds under cultivation, but has been propagated since that time by cuttings.

Apparently, the plant comes from the more shaded African rain forests,[5] the specific name being in honor of the sultan of Zanzibar.

Beginning with the accidental observation that some time after cutting back the plants, segments of internodes were found upon the soil beneath, we have made a considerable series of careful observations, embracing several hundreds of operations. Summarily stated these lead to the following conclusions:

1. Young branches of *I. Sultani* which are severely injured by the attacks of greenhouse pests may be cut off from the main stem by a sharply marked abscission zone and fall from the plant. The same seems to be true of shoots which have been injured in some other manner. In some cases we have induced the abscission of a branch by passing an electric spark through the stem, but beyond a few preliminary experiments we have given little attention to this method.

2. When a large plant is removed from a shaded room and transplanted out of doors, abscission of a number of the smaller branches almost invariably occurs. The same is true if a large cutting is made and planted, without previous trimming, in a well-lighted spot.

3. If an internode be cut across at almost any point between the nodes, the portion of that internode which remains will in the course of a few days fall from the plant. This is brought about by the formation of an abscission zone immediately above the axillary bud of the leaf of the preceding node. After the section of internode has fallen the surface left is generally very smooth—almost as smooth as a knife cut. Usually the cut is diagonal in direction, rather than transverse, beginning immediately above the axillary bud and extending downward and across the stem, so that a maximum amount of stem tissue is removed without injury to the bud.

4. If the operation be made very close to the subtending node abscission may apparently in rare cases not occur, the cut surface simply drying up. It is always difficult, however, to be sure that there has not been formed a separation zone which removed only a thin layer of cells on the cut surface. This is undoubtedly what occurs in many instances.

5. Almost invariably the abscission occurs above the first (most nearly terminal) node below the cut, but occasionally it may take

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[5] Engler. Sitzungsber. Kgl. Akad. Wiss. Berlin **16**: 191–211. 1900; Just's Jahresber. **28**^{1}: 389. Among the plants investigated by Burgerstein *I. Sultani*, *Ipomoea purpurea*, and *Tropaeolum Lobbianum* were the only species which flowered well in the shade.

place below this, but always above one of the more proximal nodes, never in the more median or distal regions of the internode.[6]

In some cases abscission above a node more proximal than the one immediately below the cut seems to follow an operation in which the internode was cut off very close indeed to the node, but this may also follow the sectioning of the internode at any point. When the whole of the internode below the one which was injured is cut off by an abscission zone, the axillary bud of the distal node is almost always, but not invariably, abortive. Whether this is the cause of, or the consequence of, the more proximal abscission than is normal we cannot assert.

6. After a first abscission above the subtending node another may later occur above some lower node. Possibly in this case the bud of the subtending node becomes abortive after the first abscission, but we can make no final statement on this point.

7. The separation of the segments of internode or internodes seems to be brought about by the disorganization of the tissues in a (generally) definite zone across the axis. Whether the cell walls are themselves broken down, or whether the cells are only separated, we have not been able to determine with certainty. The evidence furnished by the few microtome sections which we have made seems to favor the first hypothesis. When the section of internode falls the two surfaces are moist but usually very firm and smooth. Occasionally, however, the region in which the tissue is disorganized is more extensive and more irregular. In rare instances a considerable portion of an internode becomes soft and pulpy, the epidermis only remaining firm; possibly this is due to bacterial infection rather than to the processes by which the formation of the abscission zone is normally brought about.

8. In a number of experiments with our common native form, *I. fulva*, the cut end of the twig simply dried up. Abscission of the axis was never observed.

9. Any information concerning other species of plants in which similar phenomena occur will be very welcome, since we hope later to treat the subject more comprehensively.

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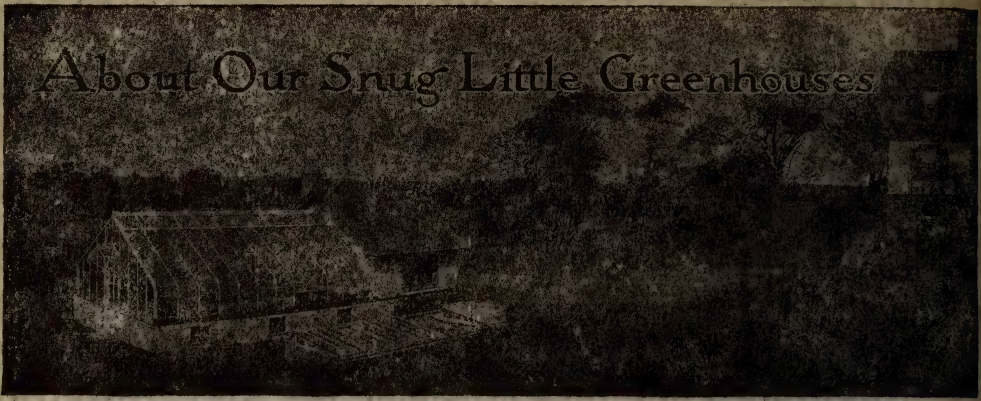
[6] We have seen one apparent exception to this rule, but the internode was in this case very short.



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ON THE MYCORHIZAS OF FOREST TREES*

W. B. McDOUGALL

I. INTRODUCTION

The study of mycorhizas has engaged the attention of numerous investigators since the appearance of Frank's (4) first paper on the subject in 1885. Altogether some sixty or seventy papers have already appeared in print dealing with the subject as a whole, or with certain phases of it. Gallaud (6) has given a very good historical résumé of the work done on mycorhizas up to 1904. It will therefore not be necessary to go into details here. Gallaud recognizes three periods in the study of mycorhizas. The first of these extends from 1840 to 1885. During this time several authors noted the presence of fungi in connection with the roots of various plants, but with very few exceptions they made no special study of them. The second period extends from 1885 to 1894, and its beginning is marked by Frank's work. Frank was really the father of mycorhiza study. He first demonstrated the true morphological nature of ectotrophic mycorhizas and applied the name to them. He also advanced the hypothesis that the fungi in question are symbiotic with the roots of the higher plants. Under the influence of this new idea numerous investigators attempted to verify or refute Frank's work, but with little success. During the third period, from 1894 to 1904, the important papers were mainly of two kinds, systematic and cytological. The systematic papers did a great deal toward extending our knowledge of the distribution of mycorhizas, while, in connection with these, and

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with the cytological papers, which dealt entirely with endotrophic mycorhizas, numerous theories were advanced as to the relation between the host plant and the fungus.

Since the publication of Gallaud's (6) extensive work on endotrophic mycorhizas, several important papers of varying nature have appeared. Bernard (1) in 1909 published his excellent work on the endotrophic mycorhizas of the orchids. This is the most complete and satisfying work yet done on the mycorhizas of any group of plants and is to a large extent verified by the work of Burgeff (2) which appeared a few months later. Other papers will be mentioned farther on.

It is a notable fact that all of the more extensive papers have dealt principally with endotrophic mycorhizas, and that no very intensive work has been done on ectotrophic forms. Mangin (13) in 1910 published a paper dealing with the ectotrophic mycorhizas of forest trees, but it is of an introductory nature, and the figures which accompany it are very diagrammatic.

The work on which the present paper is based was carried on at the University of Michigan during 1911 and 1912. The primary object from the beginning was to work out the seasonal relations of the mycorhizas of our forest trees. During the course of the work, however, a number of other interesting facts were brought to light and they will be presented here. I am indebted especially to Professor F. C. Newcombe, under whose supervision the problem was worked out, for invaluable advice and suggestive criticisms throughout the course of the work. I am also indebted to Professor C. H. Kauffman for his constant interest in the work and for assistance in dealing with the mycological side of the subject.

II. MATERIAL AND METHODS

1. Species of Trees Studied

The species of trees of whose mycorhizas the most extensive study was made are *Carya ovata* (Mill.) K. Koch, *Quercus alba* L., *Tilia americana* L., and *Betula alba* var. *papyrifera* (Marsh.) Spach., which were found to have ectotropic mycorhizas, and *Acer saccharinum* L., and *Acer rubrum* L., which have endotrophic mycorhizas. Some work was also done, for purposes of comparison, on *Larix laricina* (Du Roi.) K. Koch., *Quercus rubra* L., *Quercus velutina* Lam., *Populus grandidentata* Michx., *Fagus grandifolia* Ehrh., *Ostrya virginiana* (Mill.) K. Koch., and *Carpinus caroliniana* Walt., all of which produce ectotrophic mycorhizas, and *Acer saccharum* var. *nigrum* (Michx. f.) Britton, *Juglans nigra* L., *Crataegus* sp., and *Aesculus Hippocastanum* L., which bear endotrophic mycorhizas. No mycorhizas were found on *Cornus florida* L., *Ulmus americana* L., *Sassafras variifolium* (Salisb.) Ktze., and several species of *Salix*. These trees all occur in the woodlots in the vicinity of Ann Arbor, Michigan, or at the Forestry Farm 3 miles west of Ann Arbor.

2. Collection of Material

The collection of material was begun July 1, 1911, and continued till December, 1912. Throughout the warmer parts of the year some collecting was done every week, while during the winter collections were made less often, but specimens were obtained from each species every month, the object being to get series of specimens, extending throughout the whole twelve months, from individual trees of each species. Altogether one hundred and twenty-five collections were made. Great care was taken always to get reliable specimens. As is well known the mycorhizas occur only on the smallest rootlets of the tree and in the more superficial layers of the soil. In a mixed forest, where there are dozens of kinds of roots within a small area, it is oftentimes by no means easy to know to what tree the roots one is working with belong. In the case of a tree with the habits of Acer the task of collecting reliable specimens is not a very difficult one, because large roots of the maple often extend for considerable distances along the surface, or just beneath the surface, of the soil, where they can be easily followed. These always give off numerous short branches which can be easily traced to their ends, where mycorhizas are likely to be found. In the case of such trees as Quercus or Carya, however, it is much more difficult. On these trees the roots are usually interwoven and tangled together, so that it is very difficult to follow one for any great distance. Moreover, the large roots of these trees usually grow out only a few feet from the base of the tree when they turn downward into deeper soil, and it is more distant branches from these whose ends bear mycorhizas, often fifteen or twenty or more feet from the tree. These same trees, however, often give off short roots directly from the base of the trunk, or from the large roots near the trunk, and these very often yield an abundance of mycorhizas. When these can be found they are, of course, easily obtained. When they cannot be found, the only way of obtaining reliable specimens is to go some distance from the tree, dig until mycorhizas are found, and then, after collecting the specimens, carefully trace back the root until it is determined to what tree it belongs.

As equipment for the work of collecting I usually carry a spade, a garden trowel, and a small hunter's axe, besides a knapsack for holding a notebook and bottles for specimens. The specimens, when collected, were either put at once into 1 per cent chrom-acetic acid, or carried to the laboratory in water, and then killed in the acid. During the winter, when the soil was frozen, solid pieces of soil were chopped out with the axe, and those pieces that were thought likely to contain small roots, and, therefore, possibly mycorhizas, were taken into the laboratory and thawed out, when the mycorhizas, if present, could be picked out. Specimens collected in this way were always taken from places where the roots had been identified and their positions marked during autumn. In preparing specimens for study they were embedded in paraffin, sectioned by the microtome, and stained with gentian violet and fuchsin.

3. The Glass Plate Method of Study

Besides the method of study just outlined, the mycorhizas were studied as they grew in their natural habitat by the following method: the humus and leafmold were scraped away from a small area until some mycorhizal roots were uncovered. A round glass plate about eight inches in diameter was then placed over these roots, pressed down firmly, and covered with soil. Care must be taken that no air-spaces are left beneath the glass, and the glass must be well covered; otherwise the roots will wither and die. It is usually necessary to remove the glass when an observation is to be made.

III. OBSERVATIONS AND EXPERIMENTS

A. Ectotrophic Mycorhizas

1. *Descriptive*

Ectotrophic mycrohizas are easily recognized by the characteristic clusters of numerous, short, stubby branches which have been described as coral branching rootlets (fig. 1). When alive, they always have a bright, fresh appearance, even when collected in midwinter. They vary in color from white to bright yellow, brick red, or dark brown. They also vary greatly in the character of the external surface and internal structure. The different forms will therefore be described separately, as follows:

Form 1.—This form is found on *Carya ovata* and is, when fresh, bright yellow in color. A superficial examination with the binocular microscope, or a good pocket lens, reveals a network of mycelium over the surface and numerous short hyphae projecting out from it. A cross section of this mycorhiza (fig. 2), shows the following parts, beginning at the center: (1) The central cylinder; (2) about three circular rows of ordinary cortical cells; (3) a row of radially elongated cells. This row, however, does not extend clear around the section, but, on one side of the section, its cells are cut up into a number of irregularly shaped cells. The cells of this row are entirely separated from each other by fungus tissue which surrounds each cell on all sides. (4) Finally there is a layer of fungous filaments surrounding the section. From the outer part of this layer, which forms a mantle over the entire rootlet, tip and all, there are given off numerous filaments which project out into the surrounding soil. From the inner part of this fungus layer there are given off the filaments which surround the radially elongated cells as described above.

Form 2.—A second form, found also on *Carya ovata*, is brown in color when fresh. In microscopic section the same general parts are distinguished as in the form described above. The structural difference is concerned principally with the fungus mantle (fig. 3.) This instead of being made up of easily distinguishable filaments, consists of a pseudoparenchymatous tissue, such as is found in many lichens. The outer surface of this mantle is smooth; that is, there are no hyphae, or very few, projecting out into the soil. From the inner part of the mantle, hyphae extend inward between the cells of the root as in the former case. In the latter case, however, they often extend in farther than the single external layer of cells, and they are all divided up into short cells, so that the pseudoparenchymatous character is observed here as well as in the mantle.

Form 3.—Still another form, also found on *Carya ovata*, is white when fresh. The fungus mantle, in this case, is distinctly filamentous, but the outer surface is smooth as in the last described form (fig. 4). The root istelf and the fungus parts projecting into the root are practically of the same structure as in the first described form.

Form 4.—A fourth form found on *Quercus alba* is white when fresh. The clusters often lie more or less in one plane, as if they had been pressed, and they appear more or less wooly. The filamentous mantle, in this case, is very loosely constructed, and is easily torn and broken up while the microtome sections are being prepared.

Form 5.—A form collected from *Larix laricina* is brown in color and the thick pseudoparenchymatous mantle is smooth on the outside (fig. 6). There are no radially elongated root cells, but the outermost cells of the root have been crowded apart by the fungus until some of them are isolated as islands far out in the fungous mantle. The fungus penetrates nearly to the central cylinder so that nearly all of the cortical cells are entirely separated from each other.

Form 6.—A form found on *Tilia americana* may be called a *heterotrophic* mycorhiza. In external appearance, and in internal appearance, too, for that matter, it looks like an ectotrophic mycorhiza. It has a semi-pseudoparenchymatous fungous mantle which is smooth on the outside. It also has the radially elongated external layer of root cells, characteristic of so many ectotrophic mycorhizas, entirely surrounded and separated from each other by the fungous tissue. The novel thing about this mycorhiza is that, occasionally, the filaments, which extend in between the root cells, enter the next cells beneath, and there appear very much like endotrophic filaments (fig. 7).

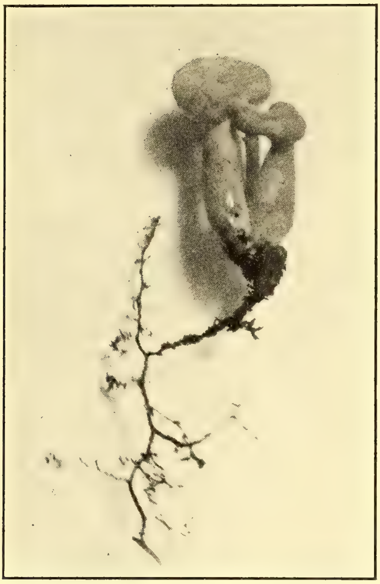
All forms of mycorhizas described above agree in containing no secondary growth. Also no fungous hyphae are ever found in those parts of a root that are not covered by the fungous mantle, so that, if two or more branches of the same root are transformed into mycorhizas, there can be no connection of the fungus of one of these mycorhizas with that of another except through the soil.

2. *Mycorhizal Fungi*

On August 6, 1912, I found the ground around the Tilia, from which I had been collecting regularly, fairly covered with the sporophores of a Russula. On digging up these mushrooms, twenty-two in number, a cluster of fresh young mycorhizas was found immediately below each one, while in three separate cases an actual connection between the mycelium of the mushroom and that of the mycorhizas was easily demonstrated (Text-fig. 1). These mushrooms were found only on the area which might be occupied by the roots of the Tilia in question. In searching over the greater part of the woods, only one other specimen was discovered. That one was near another Tilia, and when it was dug up a cluster of mycorhizas was found immediately beneath it.

I have not been able yet to determine the species of this Russula, and it may be an undescribed species. It is a small red mushroom, and it is very acrid. Evidently it belongs to the same group as do

Russula emetica Fr. and *Russula fragilis* (Pers.) Fr., yet it differs from both of these. It is not so fragile as *R. fragilis* and the cuticle does not peel off so easily. The gills are more crowded than in *R. emetica*. Most of all, it differs from these two species in the color of the spores, which in *R. emetica* and *R. fragilis* are white, while in this mycorhiza-forming species they are creamy. A peculiar thing about this Russula is that the sporophores are very apt to be deformed. Whether this is due to the presence of a parasite has not been determined. After this first crop of fruiting bodies had disappeared only four specimens were found during the rest of the season. The same form has been collected several times, however, in another locality, by Professor Kauffman.



TEXT-FIG. I. Sporophore of Russula attached to mycorhizal root.

On August 12 a dozen sporophores of *Boletus scaber* Fr. var. *fuscus* were growing under the Betula from which I had made regular collections of mycorhizas. When these were dug up, evidence was found in every case, of a connection with the mycorhizas, and in several cases an actual connection was easily seen. Later in the season another crop of these mushrooms was found in the same place, and their connections with mycorhizas were again verified. At the same time, and under the same tree, a considerable number of sporophores of Cortinarius sp. were found, and their connection with mycorhizas was demonstrated.

Finally, on the 16th of September, 1912, a fourth mycorhiza producing fungus was found, this time a puff-ball, *Scleroderma vulgare* Fr. This fungus causes the white mycorhizas which were described above as Form 4, and which occur on *Quercus alba.* A large number of the fruiting bodies were found and the pure white rhizomorphs made it comparatively easy to trace the hyphae from the mushroom to the mycorhiza.

3. *Number of Species of Fungi which may Cause Mycorhizas on the Same Tree*

The mycorhizas of Betula, caused by Cortinarius and Boletus, are easily distinguished from each other, since those caused by Cortinarius are brown, while those caused by Boletus are white. They differ also in the structure of the fungous mantle. In the Boletus-Betula mycorhizas the mantle is very compact and smooth on the outside, as in fig. 4, while in the Cortinarius-Betula mycorhizas the mantle is of a rather loose filamentous structure, and has hyphae projecting out into the soil (fig. 5).

If, now, on other trees, mycorhizas which differ greatly in color and structure are found, it may safely be concluded that they are caused by different species of fungi. Such a case was observed on *Carya ovata.* The first three mycorhizas described in this paper were all found on the same individual Carya, and differed from each other both in color and structure. Moreover, all three forms do not occur on this tree every year. In 1911 all three forms were found, the yellow form being much the most abundant; but in 1912 the yellow form was not present at all, while the white form was very abundant. The brown form was found both years in about the same numbers.

On *Quercus alba*, again, four forms of mycorhizas were found on the same tree, one of which differs from the other three in color, and all of which differ from each other in structure. Three of these are brown in color and correspond in structure to the first three forms described above. The fourth is the one which was shown to be caused by *Scleroderma vulgare*, and, since the mycelium of this fungus is pure white, it is not possible that it could be the cause of any of the brown forms. There is no doubt, then, that these four Quercus mycorhizas are caused by four different species of fungi.

4. *The Element of Chance in Mycorhiza Formation*

That the transformation of a root into a mycorhiza depends, to a large extent, on chance conditions is shown by the following observations by the glass plate method. Six plates were placed on ectotrophic species of trees, and numbered 1 to 6. Numbers 1 and 2 were on *Tilia americana;* numbers 3 and 4 were on *Betula alba* var. *papyrifera;* and numbers 5 and 6 were on *Carya ovata.* They were all placed during the month of May, 1912, and the roots under each were diagrammed on paper. Plates no. 4 and no. 5 gave no results, because air spaces were left beneath them and the roots dried up. Of the mycorhizas produced under the successful plates the longest was four millimeters and the shortest two millimeters. The lengths of the normal roots produced are given below. There was no indication in any case of the formation of a mycorhizal cluster, all mycorhizas produced being simple and unbranched. The results were as follows:

Plate No. 1, on Tilia.—Nine new rootlets were produced between June 1 and July 15. All of these had practically the same appearance, and there was no reason for supposing that their fates would be different. Four of them were dead before the end of July. The other five remained alive throughout the season. By the first of October two of these had developed normally and had attained lengths of 4.2 and 7.1 cm. respectively, while the other three had developed into typical mycorhizas.

Plate No. 2, on Tilia.—Four new rootlets were produced. Two of them became mycorhizas, and two of them developed as normal roots with lengths of 3.2 cm. and 4.1 cm.

Plate No. 3, on Betula.—Three new rootlets were produced. One of them became a mycorhiza, and two of them developed as normal roots with lengths of 2.5 cm. and 4.9 cm.

Plate No. 6, on Carya.—Three new rootlets were produced and all of them became mycorhizas.

5. *Development of a Mycorhiza*

Apparently the development of a mycorhiza takes place, in the early stages, very rapidly. Probably only a day or two is needed for the formation of a complete mantle and the development of more or less of the "lichen structure," although a considerably longer time is required before the fullest development of the mycorhiza is reached.

For this reason it is very difficult to find specimens of the early stages. The best material for this study was obtained on the seventeenth of August from *Tilia americana.* A section from some of this material is shown in fig. 10. The mantle in this case is still very thin, having the thickness of from two to four hyphae, and it is incomplete, not yet having covered the tip of the root. This shows that infection cannot have taken place at the tip, but must have taken place at some point farther back. Further, there is not yet any "lichen structure" present. In only a few places have the hyphae begun to penetrate between the cells of the root. The formation of the mantle and that of the lichen structure are going on more or less simultaneously although the mantle is in advance. The outer portion of the epidermal wall of the root can still be seen on the outside of the fungous mantle. The fungus has penetrated the epidermal wall at some point back of the root tip, split the wall by dissolving out the middle lamella, just as it does when it penetrates between the cells of the root, and spreads in all directions. Finally a complete mantle will be formed and the outer portion of the epidermal wall will be cast off entirely. Since these mycorhizas are never more than a few millimeters in length, seven or eight at most, and more often only two or three, it is evident that the root does not grow in length after a complete fungous mantle has been formed.

6. *Seasonal Relations of the Mycorhizas*

Beginning my collections about the first of July, I found that throughout the months of July and August good fresh specimens were rather scarce, although the old, black dead ones were very plentiful. Throughout October, November and December good, well-developed specimens were very abundant on all trees studied. This condition persisted during January, February and March, although these were very cold months, and the soil in which the specimens were found was frozen solid. By the middle of April the mycorhizas of Quercus and Carya had begun to die off, and good specimens were becoming rather scarce, although they were still very abundant on Tilia and Betula. By the middle of May those on the two last named species were also getting rather scarce. Throughout June and the fore part of July they were very scarce on all species. In August they began to be more plentiful again, and, by September, they were as abundant as in September of the previous year.

7. *Time of Fruiting*

As far as my observations go, it would seem that the fruiting bodies may be looked for any time after the first of August. In all cases of mycorhiza producing fungi reported in this paper, the fruiting bodies were produced relatively soon after the rootlets were infected.

B. ENDOTROPHIC MYCORHIZAS

1. *Descriptive*

The endotrophic mycorhizas of *Acer saccharinum* and *Acer rubrum* have a very characteristic appearance and are easily distinguished from the normal roots. They are constricted at intervals so that they have a beaded appearance (fig. 12). The beadlets are seldom more than one millimeter in diameter, and usually less than that. A mycorhiza may consist of only one such beadlet or there may be as many as six or more. The cortical cells in these swellings are enlarged much beyond the normal size, so that, in section, the cortical cells in the constriction appear to be greatly compressed (fig. 14). In some cases, only occasional cells contain the fungous filaments, while in other cases nearly every cell of the cortex is infected. I have never seen any of the filaments within the cells of the central cylinder. The filaments are often seen curled about the nucleus (fig. 16), but in other cases they do not appear to bear any relation whatever to the nucleus. They are often seen to pass through the cell wall from one cell to another (figs. 16 and 19), but they never split the cell walls and follow them as do the ectrotophic filaments. Peculiar swellings and various modes of branching are observed (figs. 15 and 18). The vesicles described by several authors are occasionally seen (fig. 17). These are large swellings, usually at the ends of the hyphae, and have thickened walls. The significance of these modifications is not known. Usually when only a few cells of the rootlet are occupied by the endophyte, root hairs are present in variable numbers; but root hairs are entirely lacking in those mycorhizas in which nearly all cells of the cortex are infected.

2. *The Chance of Mycorhiza Formation*

As in the case of ectotrophic mycorhizas, so here the infection of a rootlet depends on chance conditions. The glass plate method of observation did not prove as satisfactory in this case as with ectotrophic mycorhizas, but one of the plates did give some results. Under this plate, which was placed on *Acer saccharinum*, four new rootlets were produced. One of these became infected and developed into a typical mycorhiza consisting of two beadlets, while the other three developed normally and attained lengths of 2.8 cm., 3.1 cm., and 3.4 cm. respectively.

3. *The Infection of Rootlets*

A few sections were obtained in which the fungous hyphae were found within root hairs, or extending from root-hair cells into adjacent cells (fig. 19). This indicates that infection takes place through root hairs just as has been shown by several authors for other endotrophic mycorhizas.

The fungus is found only in the cortex of the beads. I have never seen it in unmodified portions of the root, nor in the constrictions between the beads. Each bead, therefore, must be infected directly from mycelium in the soil. Since the beaded appearance is only found in the case of infected rootlets, and roots which are not infected develop normally, the modified character of the mycorhiza must be due to the stimulus of infection.

4. *Seasonal Relations*

In general the seasonal relations of the endotrophic mycorhizas of Acer are the same as those of the ectotrophic mycorhizas of other forest trees. They are formed during the summer, reach their fullest development in late autumn, and die during the spring. They are, therefore, annual. They do not die off so rapidly in spring, however, as do the ectotrophic forms, and new ones are beginning to be produced long before the old ones are all dead, so that there is never a time when they are as scarce as the ectotrophic forms become.

IV. DISCUSSION

The work presented in this paper may be discussed under five heads: (1) the heterotrophic form of mycorhiza; (2) mycorhizal fungi and their relation to the chance formation of mycorhizas; (3) mycorhiza development; (4) seasonal relations; (5) physiological relations.

1. *The Heterotrophic Form of Mycorhiza*

A mycorhiza of this sort has, I believe, never been reported before. Stahl (24) reported that the mycorhiza of *Juniperus nana* is sometimes ectotrophic and sometimes endotrophic, and the one on Picea studied by

Möller (14) was usually ectotrophic but occasionally endotrophic; but neither of these authors found both forms in the same rootlet, and they had noevidence that both forms were due to the same fungus. Mangin (13) states that during the ten years he has studied mycorhizas he has never seen an ectotrophic filament within a root cell, but such a condition is found in the heterotrophic form. This mycorhiza may very well be considered an intermediate form between the ectotrophic and endotrophic mycorhizas.

2. *Mycorhizal Fungi and their Relation to the Chance Formation of Mycorhizas*

Very few attempts have been made to identify the fungi which cause ectotrophic mycorhizas, although it would seem an important work. Elaphomyces was reported by Rees (20) as causing ectotrophic mycorhizas on Pinus. Noack (16), who did more than any one else in this field, reported two species of Geaster causing mycorhizas on species of Pinus, two species of Tricholoma on Pinus and Fagus, two species of Lactarius on Fagus and Quercus, and three species of Cortinarius on Pinus, Fagus, and Quercus. Kauffman (9) has shown that *Cortinarius rubipes* Kauff. causes ectotrophic mycorhizas on *Quercus rubra* L., *Acer saccharum* Marsh., and *Celastrus scandens* L. Finally Pennington (18) has reported *Boletus speciosus* Frost and *Tricholoma transmutans* Pk. as mycorhiza formers on Quercus. With one exception, Elaphomyces, these mycorhizal fungi are all Basidiomycetes, and the four that I have added to the known list are also Basidiomycetes. On the other hand, among the identified endotrophic mycorhiza fungi there has been reported only one Basidiomycete, *Armillaria mellea*, reported by Kusano (10) as forming endotrophic mycorhizas with *Gastrodia elata*. It is quite probable, therefore, that many of the common mushrooms are capable of forming ectotrophic mycorhizas with tree roots, but that very few, if any, of them form endotrophic mycorhizas with forest trees. Moreover, all those reported are late summer and autumn mushrooms. No spring mushrooms are known to be mycorhiza formers.

The particular species of fungi and higher plants which may form mycorhizas with each other are not as limited as one might expect. MacDougal (11) states that a higher plant may form mycorhizas with only one or two species of fungi, but his observations were scarcely extensive enough to grant a positive conclusion to that effect. Kauffman (9) has shown that the same fungus may cause mycorhizas on several hosts, and my own work shows that the converse of this is true, at least in the case of ectotrophic species of forest trees.

All mycorhizal fungi, however, are not capable of forming mycorhizas with all species of mycorhizal trees. Individual trees of common mycorhiza-forming species, growing in a soil which is known to be inhabited by mycorhizal fungi, are often found entirely free from mycorhizas. A specific instance of this may be given here. In a woodlot west of Ann Arbor, two trees, a Carya and a Quercus, were found growing a few feet apart, and their roots intermingled. On the Carya, brown colored mycorhizas were found in great numbers, but on the Quercus not a specimen could be found. Apparently the fungus which formed the mycorhizas with Carya was not capable of forming any with Quercus, though this species readily forms mycorhizas. Probably the following year, or at some future time, a fungus capable of forming mycorhizas with Quercus would chance to grow here, and this same tree would then have mycorhizas.

Not only are some trees without mycorhizas likely to be found in such a habitat, but on those trees which have mycorhizas, there are always found some roots which are uninfected, even in the humus layer. This was brought out by my glass plate experiments, and is easily verified by digging up the roots of any mycorhiza forming tree. The reason for this may be that in the case of the uninfected roots, the proper fungus did not happen to be present in the immediate vicinity at the time when the root was susceptible to infection; or there may have been some physiological reason why the roots were not infected. We have no evidence either for or against the latter hypothesis; there is some evidence in favor of the former. In collecting mushrooms it is often noticed that a great abundance of specimens of a certain species may be found in a particular habitat one year, and the next year there may be none at all there. If the mycelium of this mushroom happens to be colored, so that it can be easily found in the soil, it can usually be demonstrated that when the fruiting bodies are abundant the mycelium is also abundant, while if no fruiting bodies are found the mycelium will also be lacking, or will be present only in small amounts. Now, when the mycelium of a fungus, of the proper kind, is in the soil around a tree in great abundance, the chances of the two organisms coming together and forming mycorhizas are very great. On the other hand, if the mycelium is present only in small amounts, fewer mycorhizas will be produced and more roots will develop normally. The same explanation may be applied to the fact that the same tree may have mycorhizas one year and the following year may have none at all. This fact was brought out by Kauffman (9), and has been verified by me in several instances.

3. *Mycorhiza Development*

Frank (4) stated that an ectotrophic mycorhiza is produced by a fungous filament applying itself to the side of a rootlet, and then branching and spreading until it covers the whole rootlet. He evidently believed that the mantle of fungous tissue is put on first, and that the formation of the lichen structure within the rootlet is a secondary process. These statements, however, were probably mere guesses, with no direct evidence to back them. Mangin (13) does not make himself very clear on this question. Judging from his figures, one gets the idea that the fungous mantle is put on over the tip of the rootlet first and progresses from that point until it covers the rootlet. Möller (14), on the other hand, reports, for the mycorhizas of Picea, that infection takes place back of the root tip, and that the lichen structure within the root is formed first, and the mantle put on later. Möller does not give any figures to enforce his statements, and it is of course impossible to judge of the accuracy of his observations. In the course of my work I have never seen any evidence of either of the methods of development indicated by Möller and Mangin, and the method given by me in this paper is certainly correct for the mycorhizas which I have studied.

Möller also reported that the growth of a mycorhiza is resumed in spring after a winter rest, basing his conclusions on the fact that specimens are found in spring with bright, fresh appearing tips. He therefore agreed with Frank that the mycorhiza as a whole grows in length. Such specimens with fresh tips were common on all ectotrophic trees studied by me, in the spring of the year. A number of the mycorhizas, which were already present when my glass plates were first placed, showed this characteristic. These were carefully measured from time to time during a month, at the end of which time they were all dead. In none of them was I able to discover any appreciable increase in length. It appears, then, that the fresh tips are due merely to a freshening up of the mycelium.

The fact that the root is inhibited from further growth by the fungus, offers an explanation of the coral branching clusters of mycorhizas. It is well known that when the growing tip of a shoot is cut off, or otherwise inhibited from further development, the shoot is stimulated to excessive branching. If the growing tip of a root, therefore, is inhibited from further development by a mycorhizal fungus, it is reasonable to expect that branching will result, and, if the branches are in turn infected by the fungus, the ultimate result will be a coral cluster of mycorhizas.

In regard to the development of endotrophic mycorhizas, little can be said. We have shown that infection is necessary to the production of the beads of Acer mycorhizas, and that each bead is produced by separate infection from the mycelium in the soil. Future work must show whether several beads on the same root may be produced simultaneously, or whether they must be produced consecutively. Shibata (22) found well developed nodules on the roots of Podocarpus which were uninfected by the endophyte, and concluded that infection is not necessary to the production of nodules. It has since been shown by Spratt (23), however, that the nodules of Podocarpus are due to a bacterium which inhabits them, and that the endophytic fungus is of secondary importance.

4. *The Seasonal Relations of Mycorhizas*

Möller (14) is the only previous worker who has made any serious attempt to study the seasonal variations of mycorhizas. He worked with one and two year old seedlings of Picea. His results agree with mine so far as the time of mycorhiza formation and development is concerned. He seems, however, to have missed the fact that the *mycorhizas are annual.* To sum up the results of my observations, it may be said that new mycorhizas are formed throughout the summer months. They reach their fullest development in late autumn, and persist in this condition throughout the winter. In late spring they die. They are, therefore, annual. No doubt the character of the season may hasten or retard the death of old mycorhizas and the formation of new ones, so that these processes may take place earlier some years than others. Since the formation of new mycorhizas usually begins before all of the old ones are dead, there is no time of year when no specimens at all can be found. The best time to collect for demonstration purposes, or for class use, is in late autumn just before the soil freezes up, since they can be found most easily and abundantly at this time.

Cannon (3) found that certain desert perennials annually produce a crop of fine roots, which he calls deciduous roots, at the beginning of the rainy season. During the dry season these roots all die. The annual mycorhizas really represent a second kind of deciduous roots. It must be pointed out, however, that, in the case of the mycorhizas, it is the infection by the fungus which determines whether a particular root shall become deciduous or shall develop into a perennial root, while the roots described by Cannon are all deciduous.

5. *Physiological Relations*

The question as to whether mycorhizas represent symbiotic associations or parasitic associations has been much debated, and more or less evidence, applying to particular cases, has been presented on both sides. It is worth while in the first place to consider just what the difference is between symbiosis and parasitism. By parasitism is usually understood a condition in which one organism obtains nourishment at the expense of another living organism. Symbiosis has been defined as a condition in which two organisms live in intimate relationship with each other in such a way that both are mutually benefited by the association. In the case of plants it is usually taken for granted that this benefit has to do with obtaining food. In a case of perfect symbiosis, therefore, the two symbionts must get equivalent amounts of food from each other, and the interchange must be brought about in such a way that neither plant is harmed, by a loss of a particular kind of food, more than the other. It is probable that such an ideal condition never exists in nature. We cannot, of course, suspect any symbiont of altruistic motives in supplying another plant with nourishment. Plants do not give food to each other: they take food from each other. In other words, each symbiont is a parasite on the other, and the difference between parasitism and symbiosis is only relative. Symbiosis is a double parasitism; a constant struggle for supremacy between two organisms. It is obvious, then, that a sort of association may exist which in some cases would rightly be called parasitism, and in other cases would be called symbiosis, depending on the relative potency of the individuals concerned.

There are several hypotheses which might apply to the mycorhizas: the higher plant may be parasitic on the fungus; the fungus may be parasitic on the higher plant; the association may represent a true symbiosis; or the individual cases may vary from symbiosis to parasitism.

Frank (4) first advanced the hypothesis that the ectotrophic mycorhizas represent symbiotic associations in which the fungus serves as a conveyor of food from the humus to the root. He really had no direct evidence of this, but conceived the idea principally because of the apparent similarity to the lichens. This comparison of the tissues of the mycorhizas to those of a lichen is very apt, provided that we recognize the vital differences between the two organisms. If the individual cells of the root were all separate plants, the similarity of the mycorhiza to a lichen would be very striking, but those cells are merely minute parts of a complex organism. As soon as a single cell is separated from a root, it becomes a helpless, functionless body, and soon dies. Yet that is exactly what the mycorhiza forming fungus does to the cortical cells of the root. We have shown that in all well developed ectotrophic mycorhizas the external cells of the cortex are entirely surrounded and separated from each other by the fungus hyphae, while, in some cases, nearly all of the cortical cells are similarly involved. These cells, which are thus separated from each other and from the root, can certainly never function again as root cells. The root, therefore, cannot get anything from the fungus through these cells. The only way it could get anything from the fungus, in such cases, would be by the direct contact of the fungus with the central cylinder.

Stahl (24) accepted Frank's hypothesis and attempted to prove it. After a large number of observations on plants of very diverse nature, he stated that a well-developed root system, and active transpiration, accompanied by the excretion of much water, and the presence in the leaves of an abundance of starch, calcium oxalate, and nitrates, characterize mycorhiza free plants. Plants with mycorhizas, on the other hand, he found to have a less active transpiration current, and the carbohydrates in the leaves are in soluble form. Stahl imagined a great struggle to be going on among plants growing in a humus soil to obtain the requisite amount of nutrient salts. Plants with a strong transpiration current can get along very well by themselves, but those with a weak transpiration current can get the necessary amount of food only by making use of mycorhizal fungi. On a similar basis he explains the usual absence of mycorhizas in habitats which are lacking in humus but rich in nutrient salts. The struggle there is not so great and the plants do not need mycorhizas. Also, according to this hypothesis, in a habitat which is abundantly supplied with water, the transpiration current is usually rapid enough so that plenty of mineral food can be obtained without the aid of mycorhizas. On the other hand, obligate mycorhiza forming plants are to be found, says Stahl, in habitats which are rich in humus or poor in mineral salts, since, with either of these conditions present, it is difficult for the plants to get sufficient mineral food.

Schatz (21) carried out a number of cultural experiments in an attempt to prove Stahl's hypothesis with respect to the absorption of nutrient salts, but his first cultures gave negative results, and his later cultures, though giving positive results, are mostly too few in number to be convincing, and, judging from the photographs, were not very decisive.

Yet, granting that such competition among plants exists, it is not clear that it has anything to do with the formation of mycorhizas. The natural habitat of most mycorhizal fungi is in humus soil. Very few grow in the habitats that are characterized by mycorhiza free plants, and it is obvious that, if the fungi do not occur there, mycorhizas could not be formed. Moreover, the fact that healthy trees of mycorhiza forming species are sometimes found entirely free from mycorhizas, even when growing in humus soil, shows that the trees can get along very well without mycorhizas. Again, the time of year at which the mycorhizas are developed is against the hypothesis that they aid the higher plant in the absorption of mineral salts from the soil. The mycorhizas are developed and the fungi concerned are most active during late summer and autumn. At this time of the year the trees are relatively inactive and do not need so much food as earlier in the season, when the mycorhizas are absent. On the other hand, it has been shown by Preston and Phillips (19) that there is more food stored in roots, and so available for the fungus, at this time of year than in spring and early summer; and this is just the time when the fungus needs an abundance of food, for it is preparing to produce its fruiting bodies.

There seems to be no good evidence, then, that the root gets any food through, or from, the fungus, the evidence indicating, rather, that it does not. On the other hand, there is no question but that the fungus gets some food from the root. Just how it does this is not known, since no haustoria penetrating the cells have ever been found, but it undoubtedly gets something of value from the middle lamellae of the cell walls, which it dissolves, and, since the nutrient solutions of the cell sap pass from cell to cell through the walls, the fungus has an opportunity to obtain food from that source.

We are forced to the conclusion, therefore, that the tree is not benefited by association with the fungus, and that the ectotrophic mycorhizas are not symbiotic associations, but are instances of the parasitism of fungi on the roots of trees. Usually this parasitism is comparatively harmless, although the fungus kills all rootlets which it attacks. On the average tree only a small percentage of the roots are transformed into mycorhizas, since the mycorhizas are present only in the superficial layers of the soil and all the deeper roots are allowed to function normally. For this reason the tree can endure the mycorhizas without any serious inconvenience, just as it can endure a considerable number of insect galls on its leaves. In the case of seedlings, however, the mycorhizas may become quite serious. The roots of a seedling are all in the superficial layers of the soil and, therefore, subject to the attacks of fungi. If all the rootlets are transformed into mycorhizas, the supply of mineral salts will be cut off from the seedling and it will be killed. Such a case has actually been reported by Nadson (15). The seedlings of Quercus studied by him were dying off, and he was unable to find any possible reason for their death except the ectotrophic mycorhizas which were present in unusual abundance.

In the case of endotrophic mycorhizas the situation is more complex, because the mycorhizas of different species of plants differ so greatly. No doubt an important reason why so many hypotheses have been offered to explain the physiological relations of endotrophic mycorhizas is that the different authors worked with very different sorts of plants, and the same hypothesis could not apply to all. Bernard (1) and Burgeff (2) have shown conclusively that the seeds of certain orchids will not germinate except in the presence of their mycorhizal fungi. This fact, of course, proves the symbiotic nature of the orchid mycorhizas studied by these men, but it would be unsafe to draw any conclusions from this with respect to other families of plants, or even to all orchids. Magnus (12) demonstrated just as conclusively that in the endotrophic mycorhizas of Neottia certain cells of the root digest the fungus hyphae, and so derive benefit from the association. Shibata (22) later found the same thing to be true of Podocarpus mycorhizas. Groom (7), who did his work on Thismia a few years earlier, failed to find that the hyphae were digested by the host cells, but he did find evidence that the root obtained food in some way from the fungus, and it is probable that a repetition of the work would show that digestion does take place. No cytological work has been done on the Acer mycorhiza, but it must be said that the endophyte in these mycorhizas is very similar to that described by Shibata, and acts much the same, so that it is quite possible that the same physiological processes occur.

Janse (8) advanced the hypothesis that the endophytes of endotrophic mycorhizas have the ability to fix free nitrogen, just as do the bacteria in leguminous tubercles. He had no direct evidence of this, and the few cultural experiments which he performed gave negative results, but his statements induced other workers to take up the problem from that point of view. Nobbe and Hiltner (17) performed very extensive cultural experiments in which they grew plants of Podocarpus, both with and without mycorhizal nodules, in nitrogen-free sand, and obtained results which were very favorable to Janse's hypothesis. These results, however, can no longer hold, since Spratt (23) has shown that nitrogen fixing bacteria inhabit the same nodules. Later Ternetz (25) found nitrogen fixation in cultures of fungi which she obtained from endotrophic mycorhizas, but since her method of isolating the fungi, by allowing them to grow out of the roots in hanging drop cultures, is open to criticism, the work should be subjected to verification before final acceptance. It can at least be said there is no evidence that would warrant us in suspecting the Acer endophytes of having nitrogen fixing ability.

Stahl's hypothesis of the absorption of salts from the soil certainly could not apply to the Acer mycorhizas, since there are very few communications of the endophyte with the soil. Also, these mycorhizas are not always accompanied by a low water supply, since I have collected well developed, living mycorhizas from the mud at the bottom of a kettle-hole which is full of water during all wet seasons. Not only is it impossible that the endophyte is of importance in the absorption of salts, but the rootlet itself is inhibited from absorbing them to any great extent, since it is deprived of root hairs. The endophyte, of course, gets all, or a large percentage of its food from the root. It must, therefore, be considered as an internal parasite. It is possible, however, that, in those cases, in which the endophyte occupies only a small percentage of the cortical cells, the root may receive sufficient benefit from the digestion of fungous hyphae to justify applying the term symbiosis to the association.

SUMMARY.

1. Six forms of ectotrophic mycorhizas, including a heterotrophic form, are described.

2. Four species are added to the known list of ectotrophic mycorhiza forming fungi: *Russula* sp. on *Tilia americana*, *Boletus scaber* var. *fuscus* on *Betula alba* var. *papyrifera*, *Cortinarius* sp. on *Betula alba* var. *papyrifera*, and *Scleroderma vulgare* on *Quercus alba*.

3. At least four, and probably more, different species of mushrooms may form mycorhizas on the same tree.

4. The infection of a young root and its transformation into a mycorhiza depend on the chance presence of a fungus which is capable of forming mycorhizas with that particular species of root, or on some chance condition of the root or the fungus.

5. Infection for the formation of ectotrophic mycorhizas takes place by a fungous filament penetrating the outer portion of the epidermal wall of the root and then branching and spreading in all directions, by dissolving the middle lamellae, until a complete mantle, covering the rootlet, is formed. While this mantle is being formed, other branches of the mycelium are penetrating between the epidermal cells of the root by dissolving the middle lamellae and splitting the cells apart.

6. As soon as a complete mantle of mycelium is formed over the root any further growth of the root in length is inhibited. Because of this fact the root is stimulated to produce branches. These branches are in turn infected, and the result is a coral like cluster of mycorhizas.

7. The fruiting bodies of an ectotrophic fungus are usually produced soon after the mycorhiza is formed.

8. Both ectotrophic and endotrophic mycorhizas are normally annual. They are formed during summer, reach their fullest development in late autumn, persist unchanged throughout the winter, and die in spring.

9. The roots of Acer are infected through root hairs in the production of endotrophic mycorhizas. The modified bead-like character of the mycorhizas is due to the stimulus of infection.

10. The matter presented in this paper indicates that the endotrophic mycorhizas of the maples are sometimes symbiotic associations, and sometimes associations in which the fungus can only be considered as an internal parasite of the roots. The ectotrophic mycorhizas of

forest trees, on the other hand, are not in any sense symbiotic associations, but must be considered as instances of the parasitism of fungi on the roots of trees.

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EXPLANATION OF FIGURES IN THE PLATES IV–VII

All figures, except 1, 11, and 12, were drawn with camera lucida.

FIG. 1. A root with ectotrophic mycorhizas.

FIGS. 2–6. Cross sections of ectotrophic mycorhizas. Fig. 2, form 1, from Carya ovata. Fig. 3, form 2, from Carya ovata. Fig. 4, form 3, from Quercus alba. Fig. 5, form 4, from Quercus alba. Fig. 6, form 5, from Larix laricina.

FIG. 7. Portion of cross section of heterotrophic mycorhiza from Tilia showing hyphae (*a*) which have entered the cortical cells of the root.

FIG. 8. Longitudinal section of ectotrophic mycorhiza of Quercus showing root tip covered by fungous mantle.

FIG. 9. Enlarged section of ectotrophic mycorhiza of Quercus alba showing hyphae (*a*) penetrating between the cortical cells of the root.

FIG. 10. Section of immature ectotrophic mycorhiza of Tilia americana showing root tip still uncovered and hyphae (*a*) just beginning to penetrate between the root cells. *b* = outer portion of epidermal wall.

FIG. 11. Diagram showing cortical root cells (*a*) surrounded by fungous tissue (*b*).

FIG. 12. Root with endotrophic mycorhizas.

FIG. 13. Tangential section of endotrophic mycorhiza of Acer rubrum.

FIG. 14. Longitudinal section of endotrophic mycorhiza showing the constriction between two beadlets.

FIG. 15. Enlarged cells containing endophyte.

FIG. 16. Cells with endophyte curled around nucleus.

FIG. 17. Cell containing a vesicle.

FIG. 18. Cell with endophyte showing swellings of hyphae.

FIG. 19. Hyphae within root-hairs.

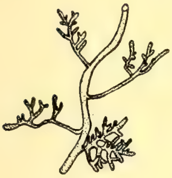

FIG. 1

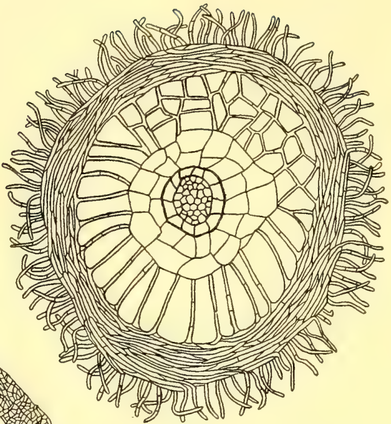

FIG. 2

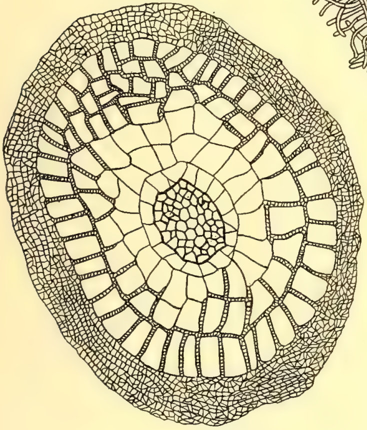

FIG. 3

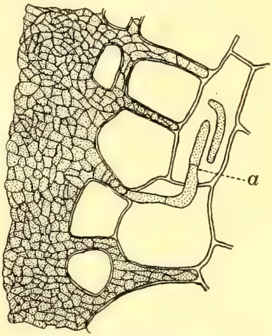

FIG. 7

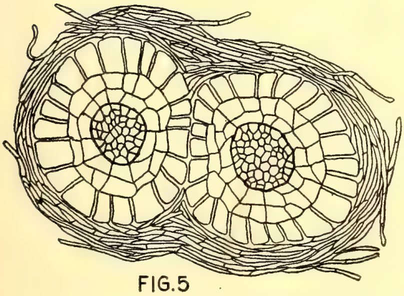

FIG. 5

McDOUGALL: MYCORHIZAS OF FOREST TREES.

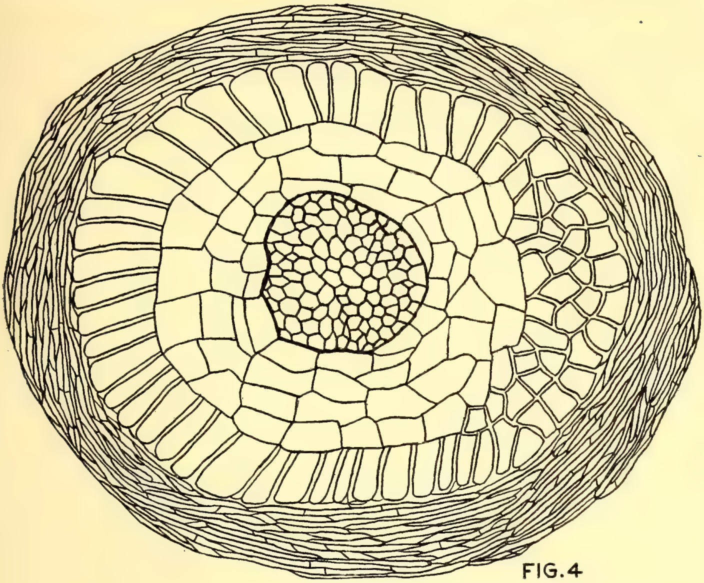

FIG. 4

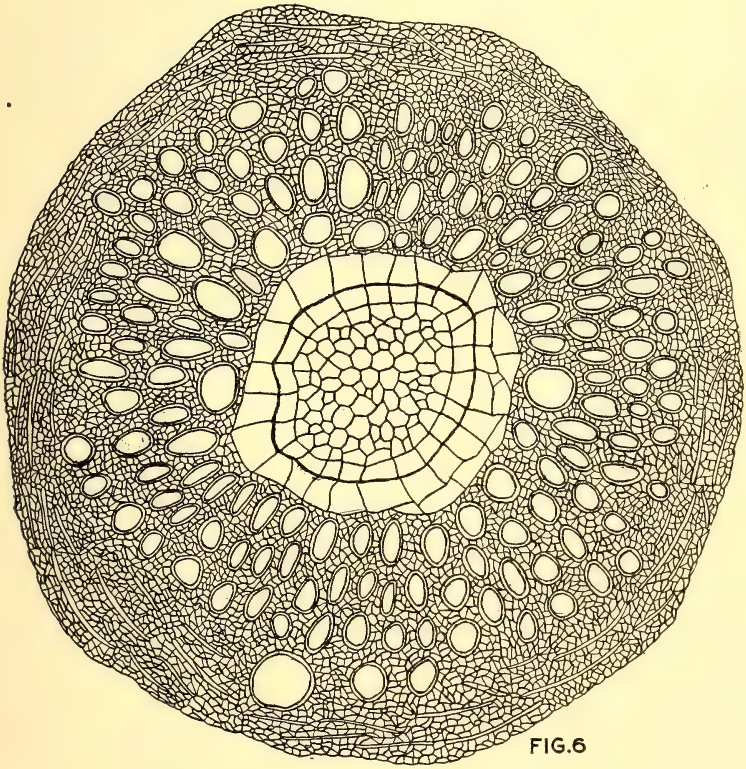

FIG. 6

McDOUGALL: MYCORHIZAS OF FOREST TREES.

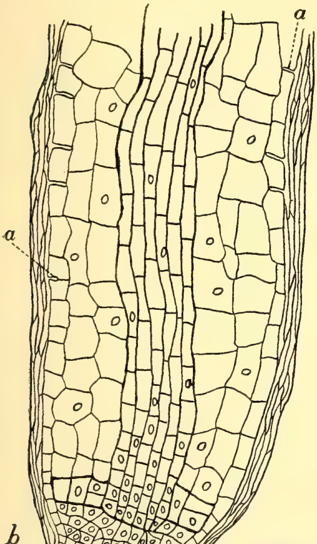

FIG. 10

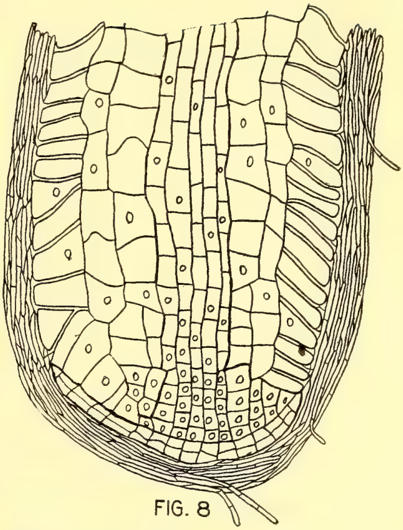

FIG. 8

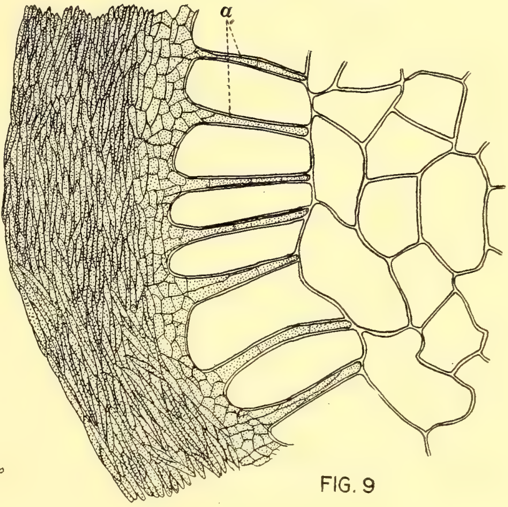

FIG. 9

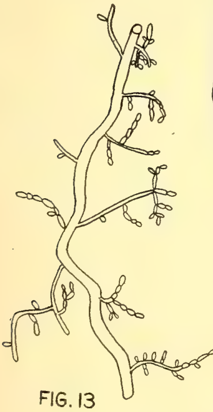

FIG. 13

McDOUGALL: MYCORHIZAS OF FOREST TREES.

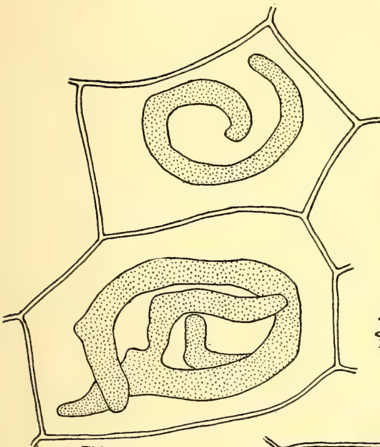

FIG. 15

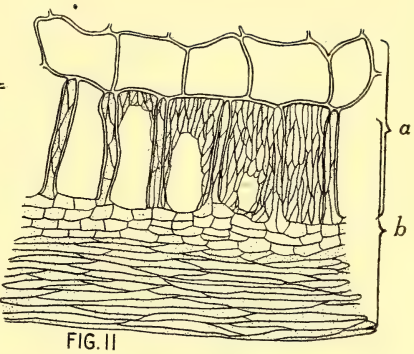

FIG. 11

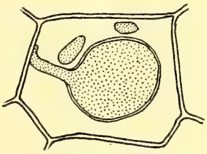

FIG 17

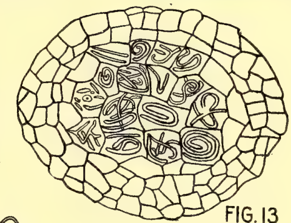

FIG. 13

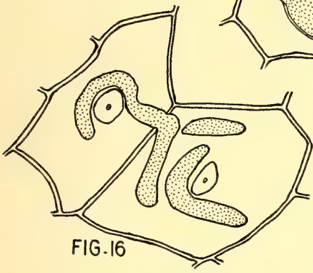

FIG. 16

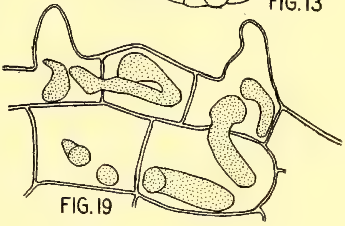

FIG. 19

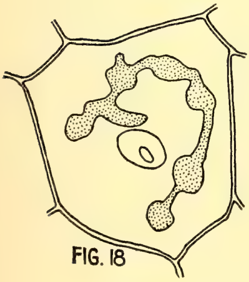

FIG. 18

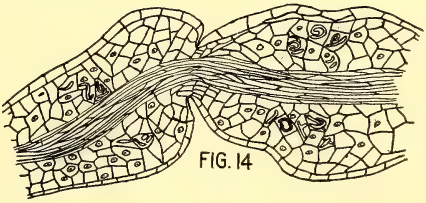

FIG. 14

McDougall: Mycorhizas of Forest Trees.

NOTES ON THE CALCULATION OF THE OSMOTIC PRESSURE OF EXPRESSED VEGETABLE SAPS FROM THE DEPRESSION OF THE FREEZING POINT, WITH A TABLE FOR THE VALUES OF P FOR $\Delta = 0.001°$ TO $\Delta = 2.999°$[1]

J. Arthur Harris and Ross Aiken Gortner

By far the simplest method of determining the osmotic pressure of expressed vegetable saps is that of the depression of the freezing point by use of the Beckmann[2] apparatus or some substitute procedure.[3] Particular emphasis has been laid upon the practicability and accuracy of the freezing point method by Lewis.[4] Renner[5] has done a good service to biologists by pointing out that inconsistencies between the plasmolytic and the cryoscopic method are, in considerable part at least, due to the difference between *weight normal* and *volume normal* solutions. Thus one objection to the cryoscopic method is removed. In this note we desire merely to call attention to an important factor which has been very generally neglected by biologists in the calculations of the osmotic pressure from the experimentally determined data, and to lighten somewhat the routine of those working in this field by the publication of a little table from which P for $\Delta = 0.001°$ to $\Delta = 2.999°$ C. may be at once determined.

The freezing point of a solution is the temperature at which ice and solution exist in equilibrium. Pure water separates upon freezing, hence the liquid which remains, and whose temperature in equilibrium with the mass of ice crystals is read from the thermometer, is not the same but is more concentrated than the original solution. In consequence the observed depression is lower than the true freezing point of the solution under investigation. That this factor may become of considerable importance in physiological work is shown at once by a comparison of corrected and uncorrected values taken from unpublished observations:

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[1] From the Station for Experimental Evolution, The Carnegie Institution of Washington.

[2] See any textbook of physical chemistry for the general methods. For some special points of technique in dealing with vegetable saps see Gortner and Harris, Notes on the Technique of the Determination of the Depression of the Freezing Point of Vegetable Saps, Plant World, **17**: 49–53. 1914.

[3] Dixon, H. H. and Atkins, W. R. G. Sci. Proc. Roy. Dublin Soc., n. s. **12**: 275–311, loc. cit. **13**: 49–62. 1911.

[4] Lewis, G. N. The Osmotic Pressure of Concentrated Solutions, and the Laws of the Perfect Solution. Journ. Amer. Chem. Soc. **30**: 668–683. 1908.

[5] Renner, G. Über die Berechnung des osmotischen Druckes. Biolog. Centralbl. **32**: 486–504. 1912. See also review of C. A. Shull, Bot. Gaz. **56**: 444. 1913.

	Δ' Depression of the freezing point as read from the thermometer	Δ Depression corrected for weight of ice separated	Difference between corrected and uncorrected depressions.
Apple	1.209°	1.166°	0.043°
Apple	1.131°	1.093°	0.038°
Pear	1.332°	1.289°	0.043°
Pear	1.461°	1.412°	0.049°
Passiflora	0.738°	0.710°	0.028°
Passiflora:	0.630°	0.607°	0.023°

Such differences cannot be neglected. They may be minimized by "salting" with a few crystals of frost after the solution has been cooled to below its freezing point, or an absolute correction may be made where the volume of the *solvent*, not the volume of the *solution*, and the amount of under-cooling (*i. e.*, the difference between the point to which the mercury fell before the separation of ice began, and the maximum temperature reached in the system, solution + ice) are known. For 1 cc. of solution,[6] volume v, having a density of d, freezing at Δ' after an under-cooling of u, and yielding total solids s, the weight of water is, of course $d - s$. Since for each degree of under-cooling 12.5 g. of water separates as ice in each liter, 1/80th of the weight of the solvent is removed and the concentration of the solution is proportionally increased. Hence the correction

$$\frac{\left(v - \frac{uv}{80}\right)\Delta'}{v} = \Delta$$

or in terms of freezing point lowering and undercooling only

$$\Delta = \Delta' - 0.0125\,u\Delta'$$

must be applied.

Some slight differences are to be noted in the literature in the method of computing the osmotic pressure in atmospheres (P) from

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[6] For simplicity of calculation merely unit-volume is adopted. In practice several cubic centimeters must be used to secure accurate figures for Δ' and s.

Δ—inconsistencies which for the most part result in variations of P less than those attributable to experimental error. In computing the accompanying table we have employed the formula of Lewis[7] quoted by Morgan.[8] To quote from Lewis[9]

> "The osmotic pressure may be obtained at once from the freezing point by means of the equation
>
> $$\pi = 12.06\Delta - 0.021\Delta^2,$$
>
> where π is the osmotic pressure in atmospheres and Δ is the lowering of the freezing point in centigrade degrees. From this equation the osmotic pressure of any solution up to 10 or 15 times normal may be obtained with an accuracy which depends only upon the precision of the freezing point determinations and upon the accuracy of the value used for the heat of fusion of ice. Since the error in the latter quantity is probably not more than 0.1 per cent, it is obvious that except for the most dilute solutions osmotic pressures may be found in this way with an accuracy which is more than ten times as great as Morse and Frazer claim for their direct measurements."

The osmotic pressures corresponding to depressions of hundredths of degrees may be read directly from the table. Furthermore *for all practical purposes* the first differences × 0.1 may be taken as 0.012, hence pressures when Δ is read to thousandths of a degree may be at once determined. Thus suppose Δ to be 1.244°. For $\Delta = 1.24°$ $P = 14.92°$. $14.92 + (4 \times 0.012) = 14.968$. The exact value by direct computation is 14.970. Or again, $\Delta = 1.248°$; $P = 14.92 + (8 \times 0.012) = 15.016$ as compared with the exact value 15.018.[10]

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[7] Lewis, G. N. Loc. cit.

[8] Morgan, J. L. R. "Elements of Physical Chemistry," 4th ed., 1908, p. 180.

[9] Lewis, G. N. Loc. cit., pp. 670–671.

[10] Since the biologist's error in the determination of Δ may be at least 0.001° C. which affects P in the second decimal place, it is idle to write down the pressures to more than four significant figures. The entries in the tables were, of course, calculated to a sufficient number of places to avoid the possibility of arithmetical error. In copying off the table the final figure has been modified where needful.

Table of Osmotic Pressures in Atmospheres for Depression of the Freezing Point to 2.999° C.

Δ	Hundredths of degrees, centigrade									
	0	1	2	3	4	5	6	7	8	9
0.0	0.000	0.121	0.241	0.362	0.482	0.603	0.724	0.844	0.965	1.085
0.1	1.206	1.327	1.447	1.568	1.688	1.809	1.930	2.050	2.171	2.291
0.2	2.412	2.532	2.652	2.772	2.893	3.014	3.134	3.255	3.375	3.496
0.3	3.616	3.737	3.857	3.978	4.098	4.219	4.339	4.459	4.580	4.700
0.4	4.821	4.941	5.062	5.182	5.302	5.423	5.543	5.664	5.784	5.904
0.5	6.025	6.145	6.266	6.386	6.506	6.627	6.747	6.867	6.988	7.108
0.6	7.229	7.349	7.469	7.590	7.710	7.830	7.951	8.071	8.191	8.312
0.7	8.432	8.552	8.672	8.793	8.913	9.033	9.154	9.274	9.394	9.514
0.8	9.635	9.755	9.875	9.995	10.12	10.24	10.36	10.48	10.60	10.72
0.9	10.84	10.96	11.08	11.20	11.32	11.44	11.56	11.68	11.80	11.92
1.0	12.04	12.16	12.28	12.40	12.52	12.64	12.76	12.88	13.00	13.12
1.1	13.24	13.36	13.48	13.60	13.72	13.84	13.96	14.08	14.20	14.32
1.2	14.44	14.56	14.68	14.80	14.92	15.04	15.16	15.28	15.40	15.52
1.3	15.64	15.76	15.88	16.00	16.12	16.24	16.36	16.48	16.60	16.72
1.4	16.84	16.96	17.08	17.20	17.32	17.44	17.56	17.68	17.80	17.92
1.5	18.04	18.16	18.28	18.40	18.52	18.64	18.76	18.88	19.00	19.12
1.6	19.24	19.36	19.48	19.60	19.72	19.84	19.96	20.08	20.20	20.32
1.7	20.44	20.56	20.68	20.80	20.92	21.04	21.16	21.28	21.40	21.52
1.8	21.64	21.76	21.88	22.00	22.12	22.24	22.36	22.48	22.60	22.72
1.9	22.84	22.96	23.08	23.20	23.32	23.44	23.56	23.68	23.80	23.92
2.0	24.04	24.16	24.28	24.40	24.52	24.63	24.75	24.87	24.99	25.11
2.1	25.23	25.35	25.47	25.59	25.71	25.83	25.95	26.07	26.19	26.31
2.2	26.43	26.55	26.67	26.79	26.91	27.03	27.15	27.27	27.39	27.51
2.3	27.63	27.75	27.87	27.99	28.11	28.23	28.34	28.46	28.58	28.70
2.4	28.82	28.94	29.06	29.18	29.30	29.42	29.54	29.66	29.78	29.90
2.5	30.02	30.14	30.26	30.38	30.50	30.62	30.74	30.86	30.98	31.09
2.6	31.21	31.33	31.45	31.57	31.69	31.81	31.93	32.05	32.17	32.29
2.7	32.41	32.53	32.65	32.77	32.89	33.00	33.13	33.25	33.36	33.48
2.8	33.60	33.72	33.84	33.96	34.08	34.20	34.31	34.43	34.56	34.68
2.9	34.79	34.91	35.04	35.16	35.27	35.39	35.51	35.63	35.75	35.87

THE PYRENOID OF ANTHOCEROS

F. McAllister

University of Texas

The single chloroplast of the cells of the gametophyte of the Anthocerotes, with its pyrenoid-like central region, has long been known. Notwithstanding this, detailed descriptions of these chloroplasts and pyrenoids and comparisons with those of the algae, on the one hand, and with the chloroplasts of the liverworts on the other, seem to be lacking.

As early as 1851 von Mohl (14) called attention to the fact that there were "wohl 50 bis 100 Amylumkörner" in the chloroplasts of Anthoceros, but he did not relate them to the starch aggregations to be found in the green algae.

The voluminous earlier literature on the morphology of Anthoceros, as far as I can determine, contains only bare mentions of the chloroplasts and pyrenoids. Leitgeb (9) in fact seems to have made no reference whatever to these structures.

Schimper (18) states that the pyrenoids of Anthoceros "zeigten nach Entfernung der Stärke durch Verdunklung nur noch corrodierte, unregelmässig eckige Umrisse." His figures 15 and 16 are apparently surface views of entire, living cells and show only diffuse central regions which have no resemblance to the plastids as seen in stained material.

Davis (5) in an account of the nuclear division and the fission of the chloroplasts in the spore mother cells of *Anthoceros laevis* makes no reference to a pyrenoid and nothing in his figures suggests such a structure. He is unable to identify plastids in the archesporial cells and only as these cells become spore mother cells can the single, very minute chloroplast be identified. This chloroplast enlarges rapidly and undergoes two fissions, thus forming the four chloroplasts of the spore tetrad. The large chloroplasts of the mature spores are filled with conspicuous starch grains, becoming thus "storage vesicles of starch."

Campbell (3, 4) has made the most important recent contribution to our knowledge of the chloroplasts of the Anthocerotes. He has shown that in certain tropical Anthoceros forms more than one chloroplast is usually found in the cells of the gametophyte and that in those cells showing the greatest increase in the number of the chloroplasts the pyrenoids are poorly defined or lacking. Referring to a species collected in Buitenzorg in which all of the interior cells of the thallus showed multiple chloroplasts, not infrequently as many as eight in a cell, he says: "The pyrenoid, usually so conspicuous in the chromatophores of the Anthocerotes, seems to be quite absent and in this respect, as well as the increased number of the chromatophores there is a close approach to the chromatophores of the other archegoniates." He finds that in a Javanese form, which he calls *Megaceros Tjibodensis*, as many as twelve chloroplasts are sometimes present in a single cell. In most of the cells, however, two to four were to be seen and occasionally but one. No trace of a pyrenoid could be recognized. In *Megaceros Salakensis* the chloroplasts of the upper surface of the thallus are larger and have aggregations of starch grains suggesting pyrenoids though less definite than in Anthoceros. The interior cells usually have four to six choloroplasts which lack pyrenoids. The author intimates that small starch grains are present in these chloroplasts (". . . nor were large starch granules observed") though he does not make a direct statement to that effect.

Sapěhin (16, 17) has shown that the presence of a single chloroplast in the sporogenous cells of Anthoceros is not peculiar to this group. He reports that the sporogenous tissues of Selaginella, Isoetes, and the mosses as well as the meristematic cells of certain Bryophytes have but a single chloroplast.

Schmitz (19) gave the name "pyrenoid" to the kernel-like bodies in the algal chloroplast which have a different texture and staining reaction than the surrounding plastid cytoplasm. Pyrenoids are found in the diatoms, certain of the Rhodophyceae, the Euglenidae, and the green algae.

De Bary as far back as 1858 (1) studied the pyrenoid in Spirogyra and determined by means of the iodin reaction that it was surrounded by an outer layer of starch, and by means of sugar and sulphuric acid claimed to show that the central part was protein.

Schmitz (19) described the pyrenoid as a comparatively dense, homogeneous mass which is differentiated from the chlorophyll-bearing protoplasm. Its microchemical reactions led him to conclude that it is similar chemically to nuclein. He describes the fission of the chloroplast and pyrenoid in Hyalotheca and believes that fission is the common mode of multiplication of pyrenoids although he is also of the opinion that they may arise from the cytoplasm *de novo.* Although recognizing the close relation of the pyrenoid to starch formation he insists that it takes no direct, morphological part in the process. He believes the starch to be deposited at all times in the clear zone immediately surrounding the pyrenoid.

The angular shape of the pyrenoid of *Bryopsis plumosa* as well as of other forms, together with the difficulty of proving its fission, led Schimper (18) to the belief that the pyrenoid is of the nature of a protein crystal. He agrees essentially with Schmitz as to the mode of the formation of starch about the pyrenoid but disagrees in part as to the mode of multiplication, believing that new pyrenoids are formed only as are new crystals, *de novo.*

Boubier (2) has proposed that not only the compact central body but also the clear area about it should be regarded as the pyrenoid. The clear area he believes to be penetrated by radiating, granular strands. Of these granular strands he says: "J'assimile cette substance granuleuse a un leucite dans les mailles duquel se depose l'amidon."

Wiesner (22) had earlier regarded the interior of the pyrenoid as made up of plastids of the nature of leucoplasts, each plastid giving rise to a starch grain.

Timberlake (20) is "inclined to the view that the pyrenoid is a active body, differentiated from the chlorophyll-bearing cytoplasm, which in co-operation with the latter acts as the basis for starch formation." His work on Hydrodictyon showed that segments split off from the pyrenoid in concentric scale-like discs. By the deposition of starch within these segments starch grains are formed which are considerably larger than the pyrenoid segment. During this process of starch formation the pyrenoid undergoes continual growth but at the same time is being reduced in size by the cutting off of rudimentary starch grains. The pyrenoid stains red with the safranin of the triple stain but at the time of the cutting off of the segments, the material cut off takes the violet stain, indicating thus its starchy nature. In Rhizoclonium, Cladophora, and Oedogonium he finds (21) that the pyrenoid often becomes split into halves and that either or both of these halves may become a starch grain, or, in some instances, the entire pyrenoid may become a single starch mass. In both of these latter cases the entire pyrenoid becomes starch.

Lutman (11) has been unable to prove with certainty the cleaving off of such rudiments of starch grains in Closterium. He finds a cleavage of the pyrenoid but is not positive that the resulting segments give rise to starch grains. He seems inclined to the view that they may give rise to new pyrenoids. His results make it clear that the pyrenoid in Closterium is not homogeneous but nearly always shows areas which absorb the stain in a varying degree. Often faintly stained lines in the pyrenoid are continuous with the clefts between the surrounding starch grains suggesting a relation between these lines in the pyrenoid and the cleavage of segments. In other cases the entire pyrenoid may break up into "lens-shaped segments."

Yamanouchi (23) has described what he regards as a new species of Hydrodictyon in the cells of which are to be seen numerous ovoid or spheroid chloroplasts. These chloroplasts "have two functions, one to produce characteristic pyrenoids and the other to form reserve starch grains." Starch formation, according to Yamanouchi, here has no relation to the pyrenoid but occurs at or near one side of the small chloroplasts, in much the same manner as the starch grains are formed by the leucoplast of Phajus. If these results are substantiated by further study we shall have the most curious phenomenon of a pyrenoid in the green algae having no relation to starch formation and the starch formed by minute chloroplasts in a manner apparently the same as in the chloroplasts and leucoplasts in the higher plants. Such evidence would seem at least sufficient to exclude this alga from the genus Hydrodictyon.

I have shown (12) that the pyrenoids of Tetraspora may split up to form several small starch grains, or the entire pyrenoid may become converted into a single starch mass much in the same manner as Timberlake (21) has described for Rhizoclonium and certain other algae.

Due to the recent researches of Lewitsky (10), Guilliermond (8), and others, a voluminous literature on the subject of chondriosomes in plant cells has been developed. The last mentioned authors claim to have established the origin of plastids from chondriosomes. It is, however, beyond the province of this paper to deal with the origin of plastids as such.

Description of Observations

The material for this study was collected in the vicinity of Ithaca, N. Y., in August, 1912. The fixation was carried on with the aid of an air pump, thus insuring quick penetration of the fixative into the intercellular cavities of the thallus. The killing agents used were Flemming's strong osmic acid mixture, medium strength osmic acid mixture as used by Strasburger, and Merkel's killing solution.

For the study of starch formation Merkel's solution gave rather the best results. The hydrogen peroxide bleaching mixture apparently exerts a weak hydrolytic action for a few days' exposure of preparations of Anthoceros, Conocephalum, Reboulia, and other liverworts containing starch to the ordinary bleaching solution (hydrogen peroxide one part and 50 per cent alcohol one part) is sufficient to remove all of the starch, or at least to render it incapable of staining either with iodin or with Flemming's triple stain. In order therefore to keep the starch as nearly as possible as it is in the living cell it is desirable to avoid the use of killing solutions which necessitate a bleaching of the fixed material. Merkel's solution answers this requirement, and other than a slight difficulty in staining achromatic figures following its use, this killing agent compares very well indeed with the osmic acid mixtures.

The chloroplast of the gametophyte of *Anthoceros laevis* is, in general, lens- or disc-shaped (figures 1 and 4). The thickness of the disc varies greatly, ranging from more than half its diameter to less than one eighth (figures 2 and 3). As will be suspected, the thicker chloroplasts are associated with abundant starch formation and are to be found in the mature areas of the thallus and close to the surface, while the flatter plastids seem to be located in cells which are more or less dormant and in the interior cells of the thick, mature thallus. Usually the surface of the disc next to the cell wall is convex while there is a tendency toward concavity on the side facing the vacuole, though a noticeable bulge is nearly always present opposite the pyrenoid.

The chloroplast varies in size according to the size of the cell in which it is situated. In the region of the growing point where the cells are small, the plastids measure about 10 microns in diameter by 4 in thickness (figure 6). In the large cells of the fully grown areas of the thallus they may be as large as 40 by 15 microns (figure 8).

Near the central part of the chloroplast, though somewhat nearer the vacuole than the cell wall, is located a more or less compact group of minute, flattened spindle- or scale-like bodies forming the so-called "pyrenoid" (figures 1, 2, and 3). An examination of a great variety of cells in various stages of activity shows definitely that there is never a homogeneous unsegmented center about which starch grains are formed as is the case with the pyrenoids of the green algae. Wherever a pyrenoid is to be seen it always appears as a group of from 25 to 300 very minute bodies rather than a single, large, spherical body. This group of bodies is often so compact as to appear practically homogeneous when viewed with a four millimeter objective although frequently, when they are not crowded, the bodies can be seen with a sixteen millimeter objective. It seems nevertheless possible that with poor fixation and staining the individual bodies might at times be unrecognizable even with an oil immersion objective.

These pyrenoid bodies, as I shall call them in the following pages, stain red or reddish with Flemming's triple stain, the brilliancy of the stain depending apparently upon the activity of the cell and plastid. In cells which are semi-dormant where growth is very slow and the pyrenoid bodies are closely crowded, they stain a dull reddish color. When loosely aggregated, as they are usually when photosynthesis is very active, they appear as flattened, scale-like bodies which stain a brilliant red. Starch grains which nearly always surround them under these conditions always take the violet stain. With Millon's reagent the central aggregation of bodies stains orange and with nitric acid the characteristic xanthoproteic reaction is obtained,—the surrounding starch grains in both cases remaining colorless. These reactions indicate as definitely as our present microchemical tests permit the protein nature of the pyrenoid bodies.

As has been suggested above, considerable difference exists in the degree of aggregation of the bodies which make up the pyrenoid. Those in the cells which contain little or no starch, or in other words, in which photosynthesis is not active, show always a compact mass of pyrenoid bodies. Figure 6 shows such an aggregation in a small cell near the growing point. The deeply lying cells of the mature thallus usually have such dense aggregations (figures 1 and 19). It is of interest in this connection to note that the peripheral cells, even on the lower surface of the thallus, always show much more active photosynthesis than those submerged in the thallus, a condition which is without doubt due to insufficient aeration of the interior regions.

In certain thalli, which are apparently in a dormant condition, practically all of the cells have these densely aggregated pyrenoids. In such cases the plastids are very thin and the pyrenoid bodies closely aggregated (figure 3).

The pyrenoid bodies in these dense aggregates are usually less angular than those in the looser, active pyrenoids. The bodies, though still of the shape of a flattened disc, have rounded edges (figures 1 and 19). Those in the active pyrenoids are loosely aggregated and are flatter, with sharp, angular edges. So loosely are they aggregated in some plastids that they can only be distinguished from the inner starch grains by their staining reaction and possibly by their smaller size (figure 8).

This difference in the shapes of the active and quiescent pyrenoid bodies shows very clearly that the shape of the bodies is not due to the mutual pressure of plastic structures in the compact aggregates, for if this were the case we should of course find the greatest angularity in the compact masses.

Due to their minuteness I have been unable to find conclusive evidence of the formation of new pyrenoid bodies by the fragmentation or fission of preexisting bodies. They however often lie very closely together and overlap in such a manner as to suggest very strongly such an origin (figure 7). Indeed, the form and the arrangement of the pyrenoid bodies and the starch grains as a whole suggests very strikingly an origin by fission. Their form is very commonly that of an elongated disc or spindle, convex on the outside and concave on the inside. The general form of the mature starch grains as well as that of the loosely aggregated pyrenoid bodies is the same as that of compact masses frequently seen among active plastids (figure 7). These compact pyrenoids among looser, active ones have apparently been slower in arriving at the proper conditions to deposit starch. Their pyrenoid bodies are brilliant red and very similar to those in active pyrenoids with abundant surrounding starch. It is difficult to conceive that bodies overlapping one another in such definite order as do the pyrenoid bodies and starch grains could have arisen *de novo* in the cytoplasm of the plastid. Nevertheless the fact of their being invisible in the very young assimilative cells in the area of intercalary growth of the sporophyte would seem to point to the conclusion that there, at least, the pyrenoid bodies are lacking entirely and later arise *de novo*.

The direct transformation of pyrenoid bodies into starch grains is easily followed. In many plastids there is a gradual transition of the color reaction, from the brilliant red of the pyrenoid bodies to the blue of the starch grains. There is no change in the shape of the bodies during this change in color though there may be a very slight increase in the size of the grains during the transition (figure 8) which is of course to be expected. There can be no doubt that the red-staining pyrenoid bodies become transformed directly into starch grains without any change in their form. After this change in the chemical composition of the bodies there is a gradual increase in the size of the starch grain until the mature grain results. During this increase in size the grain has moved outward and when it has reached the periphery its growth ceases and it slowly disappears.

The chloroplasts of the sporophyte, in the vegetative cells not shielded by the sheath of the gametophyte, do not differ in structure from those of the gametophyte, though they average considerably smaller in size. The smaller chloroplasts of the gametophyte could not be distinguished in any way from the larger sporophytic chloroplasts (figures 6 and 17).

No pyrenoids however are recognizable in the plastids of the cells of the foot, nor in the embryonic assimilative tissue of the sporophyte immediately above the foot, neither in the cells of the columella nor in the cells of the epidermis. The plastids of these cells differ also in shape, being ovoid or irregularly elongated rather than disc-shaped. In many of the chloroplasts of the cells of the region of intercalary growth large vacuoles are to be seen, while in the plastids of the spores no protoplasmic contents are to be recognized, the plastids having become, as Davis says, "storage vesicles of starch." These cells lacking pyrenoids nearly all have some starch present within them, varying from a single grain in the youngest archesporial cells to many grains in the mother cells and spores.

All of the cells of the "archesporium" contain single minute chloroplasts which are in many cases difficult to distinguish from the granular cytoplasmic cell contents (figure 9). They contain a few starch grains, but as stated above, no pyrenoid or other conspicuous bodies are to be identified. The ground substance of the chloroplast stains very slightly, appearing to have almost no granular contents. Later as these cells enlarge to form the spore mother cells, the plastids also enlarge and minute, elongated or flattened bodies (figure 10), are clearly to be seen scattered throughout the chloroplast. There is no doubt that if the "Anlagen" of these bodies were present in the plastids in the archesporial cells they were too minute to be distinguished with the highest magnifications. So far as it is possible to determine, they have arisen *de novo*. They take the blue stain and are probably starch masses. Their shape and size is essentially the same as that of the pyrenoid bodies seen in assimilative cells. Whether they ever at any earlier period stain red as do the pyrenoid bodies I have been unable to determine. That they develop directly into the large starch grains to be seen in the spores is easily proven. Figure 11 shows a chloroplast at a somewhat later period in which these bodies have enlarged greatly and are now without question starch grains. There seems thus to be no doubt that two different modes of starch formation exist in Anthoceros, the one occurring in connection with the pyrenoid, in cells active photosynthetically, and the other occurring in cells lacking pyrenoids, in which the carbohydrate is being deposited only. I have unfortunately up to the present been unable to get satisfactory material to test this experimentally.

The chloroplasts of the cells of the assimilative tissue surrounding the sporogenous layer immediately above the foot contain numerous scattered starch grains but no traces of a central aggregation of smaller bodies (figure 12). If the bodies which later aggregate to form the pyrenoid are present in these cells though scattered, they are either unrecognizable among the starch grains or they are too minute to be detected. In some of the slightly older cells of this same layer, minute bodies, which may be rudimentary pyrenoid bodies (figure 13), are to be seen scattered through the plastid. In the central region of other chloroplasts are loose aggregates of similar bodies which suggest the pyrenoid (figure 14). Along with these bodies—usually nearer the periphery of the plastid—are to be seen scattered starch grains which seem to be the same in form and structure as those seen in tissues of the sporophyte and gametophyte which are exposed to abundant light. As will be seen from figure 15 these starch grains are much larger and are easily distinguishable from the minute centrally located pyrenoid bodies. Still higher up in the region of intercalary growth the plastids show definite central aggregations of reddish stained bodies with but few or no starch grains in the peripheral region (figure 16). Higher up the form of the chloroplasts and pyrenoids becomes the same as that of those already described for the gametophyte (figure 17). Those plastids in the regions of the sporophyte receiving full exposure to the light seem to be identical with those of the gametophyte. And as is to be expected, the structure of the pyrenoid, the mode of starch formation from it, as well as the distribution of the starch, are also the same.

Thus it will be seen that all chloroplasts actively engaged in photosynthesis have the same structure. On the other hand those chloroplasts in which photosynthesis has not yet begun, as in the embryonic area of the sporophyte, and in those cells having other functions than photosynthesis as the sporogenous cells and those of the foot, the pyrenoid is lacking. In these latter cells starch is deposited in considerable amounts but not through the agency of a visible pyrenoid.

The protoplasm of the less active plastids, both of the sporophyte and the gametophyte, is usually of a compact texture showing very fine and uniform reticulation (figure 3). Plastids in which starch is being formed rapidly have a texture less uniform and regular. The reticulations are coarse and elongated in the direction of the long axes of the starch grains (figure 7). The presence earlier of starch grains in this protoplasm is no doubt responsible for this peculiarity in its texture.

The chloroplasts of Anthoceros multiply by fission as is usual with the plastids of other plants. Seen in cross section the plastid seems to elongate, and the pyrenoid to elongate with it (figure 18). The pyrenoid finally separates into two parts and we have a much elongated chloroplast with a pyrenoid in each end (figure 19). This plastid pinches in two and forms the new plastids. The chloroplast shown in figure 19 is an unusually large one found in the older part of the thallus.

As a type of the mode of starch formation in the Marchantia group, I will here refer briefly to this process in *Reboulia hemispherica*. I hope later to give a detailed account of starch formation in some of the members of this group.

The chloroplasts of the cells of this liverwort are spheroid or ovoid bodies having an average diameter of about 6 microns. The average number in each cell seems to be from 8 to 16.

The protoplasm of those plastids which lack starch is irregularly distributed. Scattered vacuole-like areas are present in which no granular protoplasm is to be detected (figure 20). In the chloroplasts which are partly filled with starch grains these clearer, homogeneous areas are less conspicuous or lacking (figure 21). No deeply stained bodies other than the starch bodies are present, either aggregated or scattered. The smallest stainable body to be detected in these plastids seems to be starch.

The starch is formed with no apparent increase in the size of the chloroplast. It is formed in disc-shaped masses which may lie in any part of the plastid and in any position. More commonly they lie near the periphery of the plastid or they may extend across the central region as flattened bi-convex discs (figures 21 and 22). Usually from three to ten of these disc-shaped starch grains are present. Plastids filled with starch have much the same general appearance as plastids to be found in the region of the foot in the sporophyte of Anthoceros (figure 12). Those of Reboulia are however smaller and have fewer starch grains. These figures of the plastids of Reboulia are very similar to the well known figures of Sachs for Funaria, both as to the shape of the starch grains and as to their distribution.

Discussion

As has been shown, the so-called pyrenoid of Anthoceros is in no case homogeneous as in the green algae but is at all times a more or less crowded mass of disc or spindle-like bodies. Lutman (11) has found that in Closterium the entire pyrenoid occasionally breaks up "into lens-shaped segments." In this segmented condition these pyrenoids may remotely resemble the multiple pyrenoids of Anthoceros. It seems quite possible however that these lens-shaped segments are indicative of the conversion of the entire pyrenoid into a number of starch grains as has been described for Oedogonium and Rhizoclonium (21) and for Tetraspora (12). In any case they are not persisting structures of the plastid as is the case with the pyrenoid bodies of the pyrenoid of Anthoceros.

On the other hand the chemical nature of the bodies making up the Anthoceros pyrenoid seems to be the same as that of the pyrenoids found in the algae. Both give positive results with microchemical tests for protein. These protein bodies serve as the foundations or the groundwork of the starch grains and are converted into starch with no change in form, and, at first, no change in size. According to Timberlake (21) the pyrenoids of Rhizoclonium, Oedogonium, and Cladophora become at times entirely transformed into a single large mass of starch. This is also frequently the case in Tetraspora (12). The mode of starch formation from these pyrenoids is then at times the same as it is in Anthoceros from the single pyrenoid bodies. It must not be lost sight of however that here the comparison is between the entire pyrenoid of the above mentioned algae and a single pyrenoid body of Anthoceros. Based upon these comparisons the pyrenoid of Anthoceros would in reality be a compact group of minute pyrenoids, that is to say a "multiple pyrenoid."

The pyrenoid of Anthoceros, then, though differing strikingly in structure from those of the green algae, has nevertheless much in common with them. The published work indicates that the structures which have previously been termed "pyrenoids," though apparently all kernel-like protein bodies, are nevertheless not all alike in their relation to starch formation. While those present in the chloroplasts of the diatoms, in certain of the Rhodophyceae, and the Euglenidae may be concerned in the formation of other carbohydrates, true starch has not been demonstrated in any members of these groups. According to Schmitz (19) and Schimper (18) the Floridian starch appearing in many of these forms seems to be formed with no apparent connection with the pyrenoids or even with the plastids. In the Chlorophyceae the work of Timberlake has shown that the pyrenoid takes a direct morphological part in the process of starch formation, at least in the forms studied by him. Segments of the pyrenoid are split off to form the rudimentary starch grains (20) or the entire pyrenoid, as cited above, may form a single large starch mass (21). And while Lutman (11) was unable to arrive at a definite conclusion as to the mode of starch formation in Closterium, his work at least offers no support to the earlier conclusions of Schmitz (19) and of Schimper (18) that the pyrenoid although closely related to starch formation, does not itself take any direct, morphological part in the process. It is probable that more weight should be attached to the more recent work because of the great advances in microscopic technique in the last thirty years.

There are no visible structures in the chloroplasts of the other Bryophyta that are analogous to the pyrenoid bodies of Anthoceros. As described above their chloroplasts are considerably smaller and do not show in any case any visible products of photosynthesis previous to the appearance of the starch grains. No bodies are visible which serve as the beginnings of the starch grains, either scattered or aggregated in a mass. The smallest stainable bodies of the plastid stain the same color as the large starch grains and are therefore quite probably starch. As in Anthoceros the size of the chloroplasts remains apparently the same whether they are free from starch or partly filled with it.

The starch in the sporogenous cells and in other cells of the sporophyte of Anthoceros which lack pyrenoids, seems however to be formed in much the same manner as in Reboulia and the other Marchantia forms. This is probably also the case with those Anthoceros species with multiple chloroplasts which lack pyrenoids, although in these plastids photosynthesis takes place while in the above mentioned plastids of the sporophyte the starch is probably deposited from soluble carbohydrate formed elsewhere.

Campbell's work (3, 4) on certain tropical Anthoceros forms would seem to indicate that some relation exists between the size of the chloroplasts and the presence of pyrenoids in them. The conditions in *Megaceros Salakensis* are especially suggestive, where the single chloroplast of the surface cells contains a poorly defined pyrenoid, while the four to six plastids of the deeper lying cells contain no pyrenoids. It is, of course, to be expected that small chloroplasts would contain correspondingly smaller pyrenoids, but their entire disappearance is unexplainable. The pyrenoid is to be seen in every cell of the gametophyte of *Anthoceros laevis* whether in the small chloroplasts in the region of the growing point or in those relatively large chloroplasts of the surface cells of active, mature thalli. Here the size of the chloroplast merely determines the size of the pyrenoid. On the other hand pyrenoids are lacking in all of the cells of the foot and the embryonic tissue of the sporophyte. Although they later become evident in the assimilative tissue they are never to be identified in the cells of the sporogenous layer. The cause of the absence of pyrenoids in the chloroplasts of the embryonic cells of the sporophyte and of the sporogenous layer may be due to a failure to form starch directly by photosynthesis in these cells.

Since Campbell's references to the pyrenoids and starch in the chloroplasts of *Megaceros Tjibodensis* and *M. Salakensis* were only incidental, his attention having been directed mainly to the grosser morphological details, it seems very much to be desired that these forms be reinvestigated to determine if possible the relation existing between the size and the number of the chloroplasts and the presence or absence of pyrenoids.

Denniston (6) has shown that in the transformation of the soluble carbohydrates to starch in the leucoplasts of Canna and Dieffenbachia and in the chloroplasts of *Pellionia Daveauana* there is a visible intermediate product which stains orange with Flemming's triple stain.

This orange stained zone he believes to be less complex chemically than the starch. He shows that this orange stain is characteristic of immature cell plates and of cell walls which are being dissolved. It is probable that at times the pyrenoid bodies of Anthoceros, as well as parts of the pyrenoid of Hydrodictyon and probably other algae, may be regarded as intermediate products of starch formation. This intermediate product reacts microchemically as protein and therefore probably has a complexity considerably greater than that of either the soluble or the insoluble carbohydrates. If it can be shown that in the case of the leucoplast the intermediate product is really a carbohydrate not greatly different from starch then it would seem that starch formation in the presence of a pyrenoid is quite a different process in which more complex reactions involving a protein stage seem to be necessary to the production of the carbohydrate. Since starch may be deposited in chloroplasts as well as in leucoplasts in the absence of light, it is quite possible that normally it is formed from soluble carbohydrate without the intervention of photosynthesis.

The phenomenon of starch formation from the pyrenoid bodies of Anthoceros seems to support the view held by Schimper (18) and Eberdt (7) that starch is formed by a transformation of protoplasm. Certainly starch which is formed by the direct transformation of a protein body can hardly be said to be secreted from the protoplasm, as is held to be the case by Meyer (13) and by Salter (15).

The formation of new pyrenoids in Anthoceros is normally by a separation of a preexisting pyrenoid into two halves during the fission of the chloroplast. New pyrenoid bodies are apparently formed similarly, that is, by the division of other pyrenoid bodies. In the embryonic tissue of the sporophyte however we have what clearly seems to be a formation of pyrenoid bodies and pyrenoids *de novo*. Formation of pyrenoids *de novo* is not uncommon in the green algae and probably should not be unexpected here. The formation of pyrenoids here is however by the aggregation of scattered pyrenoid bodies which have themselves apparently arisen *de novo*. The origin of the pyrenoid bodies from scattered parts of the chloroplast is suggestive and may indicate that their "Anlagen" have been present from the beginning of the sporophyte but scattered and not readily stainable. Such a hypothesis as this might be used to explain the formation of starch in the sporogenous cells where no visible pyrenoid is present.

SUMMARY

1. The pyrenoid of *Anthoceros laevis* is not homogeneous at any time but is made up of from 25 to 300 closely aggregated disc- or spindle-shaped bodies, which I have called pyrenoid bodies. These pyrenoid bodies give positive results with microchemical tests for protein.

2. During photosynthesis the outer bodies of the pyrenoid are converted directly into starch masses. These rudimentary starch grains increase in size to form the mature starch grains.

3. New pyrenoid bodies are formed apparently by the fission of preexisting bodies.

4. In the sporogenous tissue of the sporophyte, starch is formed in large amounts without the agency of visible pyrenoids.

5. Pyrenoids are not visible in the embryonic assimilative tissue of the sporophyte, but, as these cells are pushed away from the embryonic region, scattered bodies appear which later become closely aggregated to form the pyrenoid.

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EXPLANATION OF FIGURES IN PLATE VIII

All figures were drawn with the aid of a camera lucida. A Zeiss 2 mm apochromatic objective with an 8 compensating ocular (magnification about 1,400) was used for all figures except Figs. 7, 9, 20, 21, and 22 which were drawn with a 12 ocular.

FIG. 1. A somewhat flattened chloroplast situated in a deeply lying cell of a mature thallus. The pyrenoid is compact and is made up of bodies which stain a dull red.

FIG. 2. A large, thick chloroplast from a surface cell of a mature, active thallus. The pyrenoid is cut slightly tangentially so that not all of the bodies appear as discs.

FIG. 3. A very thin chloroplast from a surface cell of a thin thallus.

FIG. 4. A surface view of a large, active plastid. The faces of the starch grains show rather than the edges and the pyrenoid bodies appear less angular.

FIG. 5. A large plastid cut slightly obliquely.

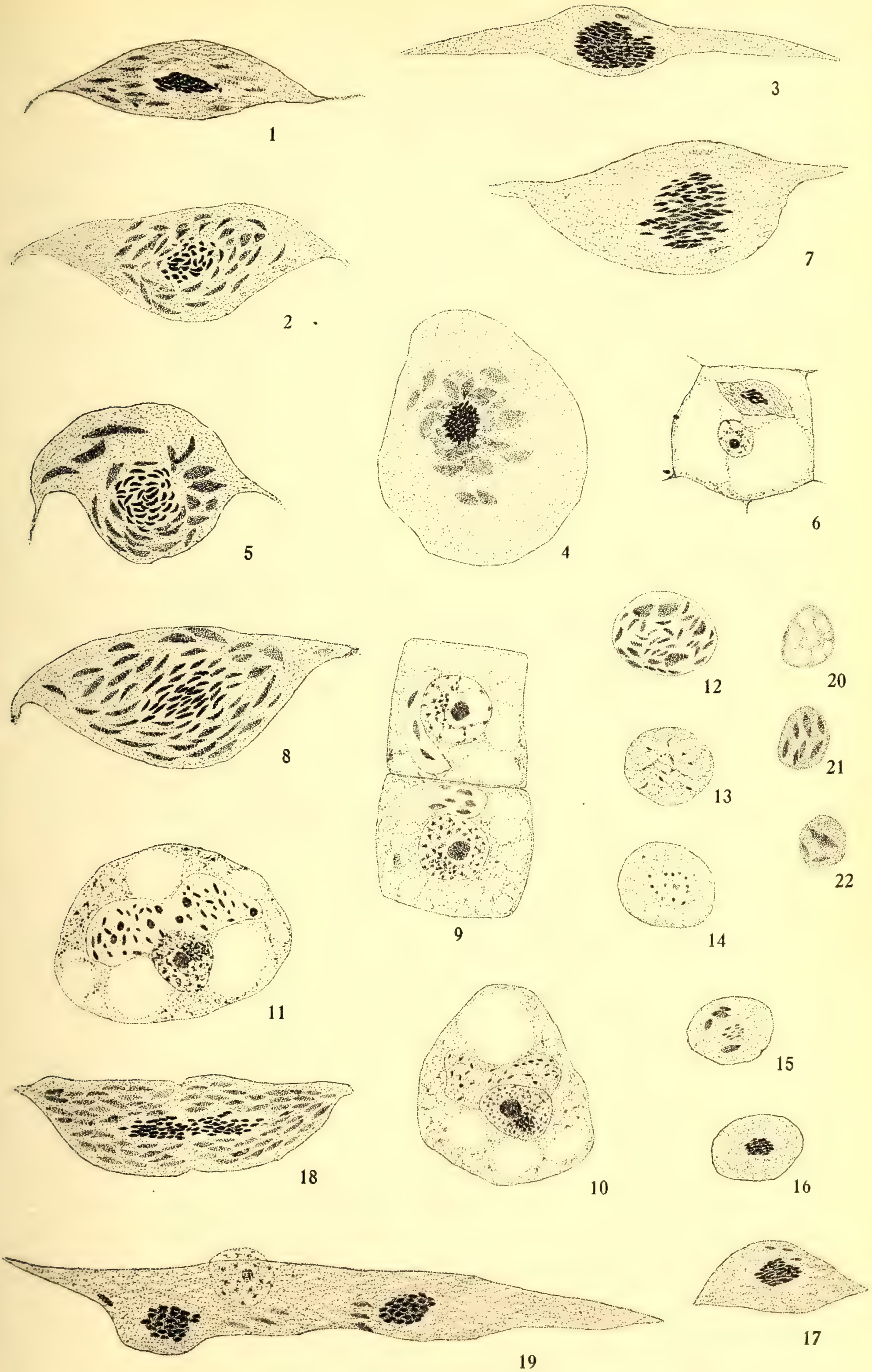
FIG. 6. A cell from near the growing point of the thallus showing the small chloroplast with its pyrenoid made up of not more than 25 to 30 pyrenoid bodies.

FIG. 7. A plastid in the vicinity of the growing point showing the pyrenoid bodies apparently multiplying by fission.

FIG. 8. A plastid in which there is a gradual transition from the loose mass of brilliantly red-stained pyrenoid bodies into the blue stained starch grains.

FIG. 9. Two archesporial cells just above the foot which show the single indistinct plastids.

FIG. 10. A spore mother cell the single plastid of which has many scattered bodies stained deeply with the violet stain. These bodies are probably very minute starch grains.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McALLISTER: PYRENOID OF ANTHOCEROS.

Fig. 11. A spore mother cell somewhat older, with the plastid filled with many small starch grains which seem to have developed from the minute violet-stained bodies of Fig. 10.

Fig. 12. A plastid in the embryonic area of the sporophyte immediately above the foot, outside the sporogenous layer. No pyrenoid is visible though the plastid is filled with starch grains showing great variation in size.

Fig. 13. A slightly older plastid from the same region as Fig. 12 which has no starch but has scattered stained bodies which may be rudimentary pyrenoid bodies.

Fig. 14. A chloroplast older than that in Fig. 13 in which a number of minute bodies are to be seen in the central region,—probably scattered pyrenoid bodies.

Fig. 15. A slightly older stage in which the pyrenoid can be identified with certainty.

Fig. 16. A plastid in the upper limits of intercalary growth which has a conspicuous dull red pyrenoid.

Fig. 17. A chloroplast somewhat older than that shown in Fig. 16, at the upper edge of the gametophytic sheath.

Fig. 18. A large plastid in a gametophytic cell, beginning to divide.

Fig. 19. A very large plastid of an interior cell of the thallus, with the pyrenoid divided but the plastid still undivided.

Fig. 20. Chloroplast of Reboulia hemispherica lacking starch.

Figs. 21 and 22. Chloroplasts of Reboulia showing the common form and distribution of the starch grains.

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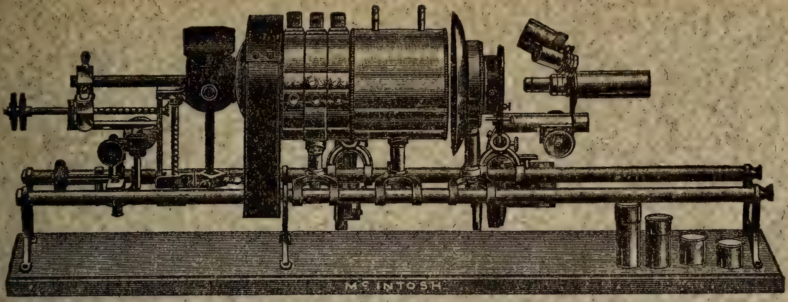

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PROBLEMS AND PROGRESS IN PLANT PATHOLOGY

L. R. Jones[1]

I. Introduction

It may be assumed, I trust, that I am doing the expected thing in choosing the topic of this address from my own field, phytopathology. If, however, justification is asked, the answer is clear. Plant pathology is simply a phase of botany. Practically all progress to date in its scientific development is owing to botanists. The rapid increase in numbers of those engaged upon work in this branch of botanical science has, however, naturally crystallized certain tendencies to segregation, giving us our independent Phytopathological Society with its separate program and its own journal. While this segregation is, in my judgment, the natural and wholesome result of progress, it creates problems and embodies danger to both parties. To the parent group, these lie in the loss of close association, heretofore had with some of its virile younger members; to the younger branch, there is the even more serious danger in passing from the critical and standardizing influence of the general Botanical Society dominated by maturer minds and broader ideals.

If we accept as true the statement of one year ago by Dr. Farlow[2] that America is today surpassing other nations in the study and applications of plant pathology, perhaps the first phase of biological science where this can be asserted, all will agree that much credit for this is

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[1] Address of the retiring President of the Botanical Society of America, read at the Atlanta meeting, Dec. 31, 1913.

[2] Farlow, W. G. The change from the old to the new botany in the United States. Science, N. S. **37**: 79. 1913.

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due to the fact that our methods, ideals and leadership have come directly from botanical circles. Now that these relations are becoming less intimate, the responsibility rests upon both parties to see that by conscious effort we keep in closest touch; that the dangers of mutual loss from segregation be minimized to the utmost.

I have chosen the combination title, Problems and Progress, because of the necessary relationship of these two ideas. There may, indeed, be difference of opinion as to the relative stage of scientific progress in plant pathology, as compared with other branches of botany. There must, however, be general agreement as to the relatively great increase in activity in this field in the last two decades. Activity is the gage of life, and fullness of life should be the best criterion of progress. But we all recognize that whether or not activity or life in any scientific field does measure progress depends upon whether or not action is directed toward the solution of fundamental problems.

Let us with this in mind review the progress in phytopathology, trying to define and delimit some of the chief problems as they have successively arisen and to decide in how far they have been solved.

II. The Problem of Parasitism

Practical-minded men have faced the problems of disease in plants since plant culture began and those more scientifically minded have, of course, speculated or investigated in the matter. But it will profit us little to go back much more than a century for inquiry into either their definition of the problems or their progress in the solution. When Count Re, of Italy (1807),[3] following the lead of the Tyrolese von Zallinger (1773), attempted an account of what was known about plant diseases, practical or scientific, the result was largely barren because he had no conception of the meaning of parasitism. Little was known about the fungi and less about their host relations. Schweinitz, Persoon and Fries soon laid the secure foundations for mycological nomenclature and species descriptions, secure because based on keen observations and critical comparisons. But they had no concern with plant pathology and their contemporaries who had were star-gazing with the nature philosophers. Thus Count Re's work remained nearly half a century after it was published as a standard writing in plant pathology.[4]

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[3] Re, Fillipo. Saggio teorico pratico sulle malattie delle piante. Veneziae. 1807. An English translation was published in Gardiner's Chronicle, 1849, p. 228.

It required the plague of the potato disease and the example of the Irish famine finally to focus attention upon the fundamental problem—the relation of the mildew to the sick potato plant, of the smut and rust fungi to the infected grain—the problem of parasitism. True, they had been phrasing the term parasite much as we do, but so long as most held that the so-called parasitic fungus originated through the transformation of the sap or the degeneration of the diseased host tissues there could be no real progress in plant pathology whether scientific or practical. To De Bary's master mind we owe the clear recognition of the parasitic relations of fungus and host plant,[5] and from his demonstration of this we date further progress.

But although De Bary's work has settled for all time that the parasite is an independent plant entering the host from without and feeding upon it to its destruction, we must not forget that the more fundamental problems of parasitism remain with us. In biology, the definition is always dangerous, and the more complete and finished the more the danger. De Bary's classification of all fungi as parasites and saprophytes, obligate and facultative, is so complete and satisfying that it is constantly misleading. De Bary thought as the mycologist with attention focused upon the fungus. The first concern of the pathologist must ever be with the host plant, and chiefly with the host plant under conditions of culture. He must constantly be alert to the fact that parasitism is not a fixed but a fluctuating relation, dependent as to its occurrence and degree upon a complex of conditions, and these involving the reactions of not one but two widely different organisms. Although the fact of parasitism was settled and the modern science of plant pathology securely based upon it, there has been no time since when phytopathologists realized as clearly as today the importance of the problems yet to be solved in this field. We have scarcely begun the study of the intimate relations of parasite and host, the conditions and results of parasitism.

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[4] The editor of Gardiner's Chronicle (1849, p. 211) prefaces the translation of Re's work with the statement that "it is the best work within our knowledge" upon this subject.

[5] De Bary, A. Untersuchungen über die Brandpilze und die durch sie verursachten Krankheiten der Pflanzen mit Rücksicht auf das Getreide und andere Nutzpflanzen. 1853.

III. THE LIFE-HISTORY PROBLEMS

The fact of parasitism accepted, the problem of the life history of the parasite at once presented itself to these early students. Kühn's work on grain infection by smut (1858) and De Bary's upon the life histories of the Peronosporales (1863) with proof of heteroecism of the rusts (1864–5) set the pace. In the retiring address of my predecessor,[6] we learned how Farlow brought the coals to this country which have kindled the fires of our best American research in mycological pathology.

It should remain the first concern of plant pathologists that this work be continued. Discoveries as to life histories of parasites are, in the long run, of more practical importance as fundamental for disease control than demonstrations with spray mixtures. The latter are usually transient contributions, the former permanent. It is, therefore, of good promise that the two life-history problems which first engaged De Bary's efforts, those of the grain rust and the potato fungus, are today held more open and are receiving more earnest attention than when De Bary died. It is well that the problem of the overwintering of the apple scab is no sooner settled by one investigator for one locality than it is opened by another, working in a different environment. Life history problems have so many variations and complexities that they must ever remain with us, and progress in their fuller solution will continue as one index to general progress in plant pathology.

It is fortunate that they are so well suited for thesis problems of graduate students, and we may hope that the traditions established in the laboratories of Farlow and Atkinson may be perpetuated as well in other institutions.

IV. THE CULTURE PROBLEMS

While De Bary in Germany was laying the foundation of mycological morphology, Pasteur in France was doing a correspondingly important work on the side of physiology, dealing with the fundamentals of fermentation and nutrition. Following his initial efforts, the problem of the pure culture with yeasts and bacteria was promptly defined and solved. Bacteriology not only came quickly into existence, but soon became the most exact science of the biological group, owing to the fact that in such pure cultures environmental conditions can be controlled to a degree unattainable with the higher organisms.

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[6] Farlow, W. G. Loc. cit.

Brefeld's success in culturing the smuts directed attention to this new method in studying the fungous parasites. Although the methods were adapted from those of the bacteriologists their uses with fungi are somewhat different. With these it is not only the gain from exact handling in differentiating mixed infections and inoculating from pure cultures, but also in completing life history investigations. With the imperfect fungi and Pyrenomycetes the method is especially applicable and the recent work on Glomerella by Shear[7] and Edgerton[8] illustrates well its advantages. To this method *Phytophthora infestans* has at last yielded the clue to its complete life history,[9] although here as always the developments in the culture tube need to be checked by comparison with those in nature.

For culturing the plant pathogens the value of the solid over liquid media and of vegetable over animal extracts becomes increasingly evident with experience. Thus the merits of Clinton's oat agar which gave such important results with Phytophthora have again been shown by the development upon this medium in our laboratory of perithecia of the apple scab fungus in greater abundance and vigor than ever observed in nature.[10] It should be assumed that for all such fungi which develop part of their fruiting stages saprophytically we may perfect culture media and methods which will not only simulate but may improve on those of nature.

And even the so-called obligate parasites deserve attention, for we are not restricted to artificial or dead media in pure culture work. The living sterile tissues of the proper host may be secured for many parasites providing only the need is sufficient to justify the painstaking. This of course, is easy with many interior tissues of fleshy parts, while for various other plants the seedlings may be grown from sterile seeds. It would seem that the problem of whether or not *Plasmodiophora brassicae* is the sole cause of club root of crucifers, or whether association is necessary with bacterial or other organisms, as has been suggested,[11] is a challenge to such increased skill in culture technique.

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[7] Shear, C. L. and Wood, A. K. Studies of fungous parasites belonging to the genus Glomerella. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 252. 1913.

[8] Edgerton, C. W. Plus and minus strains in an Ascomycete. Science, N. S. **35**: 151. 1912; also paper read at this Atlanta meeting.

[9] See Jones, L. R. and Giddings, N. J. Studies of the potato fungus. Science, N. S. **39**: 271. 1909. See also, ibid. **30**: 813, 1909; **31**: 752, 1910. Clinton, G. P. Oospores of potato blight. Science, N. S. **33**: 744, 1911; Conn. Agr. Exp. Sta. Rept. 1909–10: 753. Jones, Giddings and Lutman. Investigations of the potato fungus. U. S. Dept. Agr. Bur. Pl. Ind. Bul. 245. 1912. Pethybridge, G. H. On pure cultures of Phytophthora infestans De Bary and the development of oospores. Sci. Proc. Royal Dublin Soc. **13**: 556. 1913.

[10] See abstract of paper by F. R. Jones: "Perithecia in cultures of Venturia inaequalis." Phytopathology **4**: 52. 1914.

Finally, there is culturing upon the living host. Although this was the earliest method in vogue, and has yielded such gains especially in the hands of Arthur and others with rusts, yet the general applicability and importance of this practice in plant pathological investigations has not been fully realized. It is only thus that we can learn with exactness of related varietal or species susceptibility of hosts on the one hand, and of the occurrence of biological forms among parasites on the other—both things of paramount importance in plant pathology, scientific and economic. Success in such work is conditioned upon our ability to control and interpret environmental conditions. When the superiority of the greenhouse for such studies is more fully realized, we shall here work out the most of our fundamental problems, with the field plat as the place more important for verification than for investigation.

V. Bacteria in Relation to Plant Disease

The problems of bacteria in relation to plant disease naturally followed the advent of the pure culture method. While, from the American standpoint, this is the most important chapter in the development of modern plant pathology, it is at the same time, to us, the most familiar. The universally acknowledged world supremacy rests here, thanks to the high ideals and energetic—at times militant—leadership of him who two years ago was the honored president of this Society. I may only outline certain things in order to warn of dangers or suggest other problems.

Since the work of Burrill over forty years ago, no American worker has doubted the occurrence of bacterial diseases of plants. That Europeans were skeptical for a time was the natural consequence of too great reliance upon tradition and too great respect for authority. And as we grow older in the work in America we must realize that the traditions will soon be ours and that the paralyzing hand of authority will rest more heavily upon us. While in general we must follow its lead, and the "progressive" who breaks from the ranks must do so at his peril, let us keep alive to the need of progressiveness, and be patient with the man who challenges a traditional idea. Of course, every American recognizes fire blight of pear as the "classic" among bacterial diseases. But there may be blight which is not the bacterial fire blight. It is a wholesome thing, therefore, to have a challenge issued. It has been too easy, at least in horticultural circles of the west, to attribute all types of blighting of pear and apple trees to *Bacillus amylovorus.* One of the most reassuring things about the chestnut blight situation has been the fact that from the outset there have been those who must be converted. I have for years been convinced that American pathologists have relied too implicitly on authority in attributing all potato scab to one organism. Now that our so-called "Oospora scabies" seems to be of a bacterial nature[12] and the powdery scab of Europe is threatening if not invading our territory, we may hope for a revival of first hand investigations. And may we not be in danger of generalizing too broadly with reference to galls? The brilliancy and thoroughness of the recent work upon crown gall will almost inevitably encourage this in spite of the guarded and conservative statements made by the authors themselves.

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[11] Pinoy. Role des bactéries dans le developpement du Plasmodiophora brassicae. Compt. Rend. Soc. Biol. **58**: 1010. 1905.

The natural consequence of the general acceptance of the fact of bacterial diseases of plants, coupled with the lack of adequate training in bacteriological technique, led many in the early days to attribute numerous diseases to bacteria upon incomplete evidence. Nor was this confined to America. European literature, especially the French, has many such announcements. We need not criticize these too severely as to the past. It was natural and inevitable. But we are increasingly blameworthy if we continue either to publish carelessly or to accept the announcements of others without critical review. With the appearance of Smith's monographic work on Bacteria in Relation to Plant Diseases, any American at least, who describes a "new" bacterial disease of plants upon inadequate data should realize that he is committing an offence against the American profession.

This is not to imply that there are not plenty of bacterial diseases of plants yet to be discovered, nor to discourage the search for these. It is rather to emphasize that there are other problems better worth while than the search for "new" diseases of minor economic importance. The simplicity of the bacteria in their relations to host and in the way they lend themselves to culture and infection stimulate the hope that through persistent intensive study of bacterial diseases we shall gain the clearer insight into those intimate relations of parasite and host which are fundamental to the science of plant pathology.

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[12] Cunningham, G. C. The relationship of *Oospora scabies* to the higher bacteria. Phytopathology **2**: 97. 1912.

VI. The Relations of Parasite to Host and Environment

Although parasitology in relation to plant pathology dates from but little later than in animal pathology; and the relations involved would seem simpler than with the animal parasite, yet the fact remains that we are far behind the animal pathologists in understanding these relations. Some of the reasons for this are evident. The preeminent value of human life among the animals has focused attention upon human pathology. Even where attention has been given to the pathology of the lower animals, the students have as a rule approached the subject from the viewpoint of human pathology, and have been eager to apply to this any suggestions from comparative work on the lower forms. The result has been intensity and concentration of research upon the diseases of this one organism, man.

In plant pathology the natural tendency has been exactly the opposite. From the beginning the phytopathologist has included in his range of interests all the diseases of all plants known to him. The numbers of disease-inducing parasites is so enormous that it has consumed his professional energies simply to catalogue them. Concentration when attempted has been secured by narrowing one's interests within the parasitic group rather than within the host group.

I believe that we need to have, far more than heretofore, specialization by hosts in our phytopathological studies. Whether one is to probe deeper into problems of relations of environment to parasitism or into matters of predisposition and variations, either with host as to susceptibility or parasite as to its biological forms, attention should be focused long and intensively upon the one host. Experience has convinced me that one cannot understand the diseases of a cultivated plant like the potato, for example, except as he understands them in relation not only to the normal physiology and morphology of the plant, but in relation to its history and its variations under culture. Progress requires that we have specialists on types of host plant as well as of parasite.

And, passing to the cellular relations of host and parasite, how little we know. The very simplicity of the plant's organization makes the pathological reactions harder to investigate than with the animal. In the plant the unit in the more fundamental pathological relations is not the organism but the cell, an object so minute as to make the study of the chemical interrelations highly difficult. We recognize the cell membrane as the first barrier to be overcome by the invader and we believe the cytolytic enzyme the first weapon in the attack. Yet, save with certain soft rot diseases, we know little that is definite about these enzymes in their action. We see evidence of other disturbing effects of parasite upon host cells, even in advance of actual invasion. Sometimes these are inhibitory or fatal, sometimes stimulating. But we have scarcely sufficient basis for a suggestion as to the nature of the agents involved. Such problems call for the combined skill of pathologist, physiologist, cytologist and chemist.

The variation in the occurrence of disease with environment is one of the commonest observations and a thing of the greatest practical moment. Yet how little progress we have made in understanding the factors. Climate and soil, both are composites of many variables, which in turn may react on either host or parasite. Why is it that Rhizoctonia diseases and Blattrollkrankheit of the potato claim so much attention in certain sections of the United States while in others pathologists are skeptical as to their existence? Why is it that the bacterial black leg of the potato develops so much worse in the south than in the north? Why is it that with the melon the Fusarium wilt is the scourge of the one section and the bacterial wilt of another? Why is it that the yellows disease of cabbage exterminates the crop under certain conditions and is of minor importance under others?

It would seem that here are problems to challenge the attention of every pathologist. Yet if one turns to them he is balked at the outset. We have inadequate data as yet regarding the occurrence and distribution of even the commonest economic diseases in the United States. Let us unite in urging that in the reorganization of the work now in progress in the Bureau of Plant Industry the entire attention of at least one expert pathologist be given to collecting and analyzing such data, while all local pathologists pledge the undertaking continued support and cooperation. Coordinate with this, the local student of the special disease may make painstaking studies in field, greenhouse and culture chamber and in time delimit the effects of moisture, temperature, soil reaction and like factors upon each parasite and host.

The evidence is accumulating that the variations in relations between parasite and host which give us specialized races of parasites on one hand, and, on the other, gradations in disease-resistance of host, are of the greatest importance, whether scientific or practical. But we can as yet record little that helps us adequately to define the factors in the problems, much less to solve them.

As suggested before, these problems are at bottom physiological and of the most complex kind. The pathology of the past has been the work of the mycologist and the bacteriologist. That of the future must be increasingly dependent upon the physiologist; for what is pathology at bottom but abnormal physiology? Realizing how slow is progress upon the really fundamental problems in normal physiology and what dearth there is of workers adequately trained to grapple with them, we must be patient with ourselves and beg the patience of others when dealing thus with the abnormal. Perhaps our greatest hopes lie in the assurance that from now on increasing attention must and will be given to the training in physiology of those who are coming into the profession of plant pathology.

VII. The Non-Parasitic Disease

If the early workers in plant pathology erred in failing to recognize the importance of parasites as causal agents, the recent ones have gone to the other extreme.

The mycologist and the bacteriologist naturally bring to our attention even the minor parasitic maladies, the physiologist has as yet rarely come to our aid. It is only as one undertakes the comprehensive study of the maladies of a particular host that he realizes how few of the non-parasitic diseases have been listed.

Perhaps the peach, the tobacco and the potato are the only plants where the energies have been duly distributed between the investigations of parasitic and non-parasitic diseases. If anyone doubts that in these non-parasitic maladies we are dealing with specific diseases having clearly defined symptoms which follow a regular course, let him grow China asters for a series of years in his garden and trace the course of aster yellows.[13] Here we have a malady as clearly characterized as a fungus rust or wilt disease; unknown, I believe, in Europe, but widespread in America, variable with season and locality, yet its etiology and pathology are entirely problematical.

But these are not problems to be undertaken lightly. Considering their inherent difficulties we may be thankful that such critical and persistent work has been given to certain types already, notably to peach yellows by Smith and to the mosaic disease of tobacco by Mayer, Beijerinck, Woods and others. It is encouraging to see that earnest attention is being given to certain apple maladies in different sections, especially the so-called "brown spot" or "bitter pit" in South Africa and Australia.[14]

Our encouragement will be greater, however, when we see the clear recognition of the fact that training in parasitology has only indirect value when it comes to such problems. The most evident need if we are to advance in the fundamentals of our research in this field of plant pathology is the reinforcement of our ranks with young men equipped with a high degree of special training in plant physiology grounded in organic chemistry, and ready to dedicate their services long and patiently to these physiological researches.

VIII. The Problems of Disease Control

Now you are expecting the statistics showing how many millions America is adding to her income by modern methods in disease control; but you have heard them often, so I need not repeat them; and they have much of truth in them. The yankee is practical, and the yankee mind dominates everywhere in America. Instead of boasting we rather owe ourselves this explanation—shall we say apology—when we point to the relative proportion of the space in American plant pathological publications given to the consideration of the spray pump and the disinfecting solution. How could it be otherwise? The millions spent by patent medicine advertisers have implanted firmly in the American mind the idea that each animal disease is a specific thing and for it there exists a specific remedy. It was, therefore, most lucky that when the professional "plant doctor" was introduced to the American rural constituency by the state experiment stations and national Department of Agriculture, he could step forward with Bordeaux mixture in one hand and formaldehyde in the other, two specifics which could at once be used and misused in a most amazing variety of cases without serious danger of loss of life or reputation. And just as these were becoming somewhat commonplace lime-sulfur was brought to our aid and with it the added enterprise of the American commercial advertiser.

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[13] See Stone, G. E. and Smith, R. E. Mass. Agr. Exp. Sta. Bul. 79. 1902.

[14] McAlpine, D. Bitter pit investigations. First progress report. Melbourne. 1911–12.

Please do not misunderstand me. I recognize clearly that the highest duty in plant pathology is service, and that the chief aim in that service is to lessen loss from plant diseases. The only question is, how can we best serve to this end?

Perhaps as conditions have been, we could not at the outset have done much better. It was necessary first to educate the public as to the amount of their loss from plant diseases, as to the general nature of the parasites, and as to the great gains from the use of fungicides. In order to do this, the pathologist must familiarize himself with these things by repeated observations and trials and must contribute in turn to the education of the horticulturist, the agronomist and the agricultural press. This has taken time, in many cases nearly all of his time. But we may have satisfaction in the idea that it has been well done. No other country has had like service and in no other country has the agricultural public followed the teachings so fully.

It is important, however, for us to remember that this is the pioneer service, necessary and best at the outset; but that, as fast as conditions permit, we must be moving on to the attack on the more fundamental problems, to the performance of the more enduring service. The fundamental idea in plant disease control is prevention. It is surprising if one goes over the list how many diseases cannot be prevented by the use of fungicides. For the great classes of bacterial diseases, rusts and soil fungi, we must look to other measures. The three fundamental ideas which here deserve increased attention are sanitation, exclusion, disease resistance.

Spraying and seed treatments are only one part of sanitation in any case and have no part in many cases. Full data as to the life histories and modes of dissemination of causal organisms are more important fundamentals for improved sanitation than are further demonstrations with fungicides. The importance of fertilization, cultivation and crop rotation in relation to sanitation, together with the destruction of diseased plant tissues and the checking of the carriers of disease germs, deserve more critical attention than they have received from plant pathologists as well as plant cultivators.

While America has for some time been the most advanced nation in controlling diseases by spraying, she has been one of the slowest to undertake plant disease exclusion. The plant quarantine act secured last year by the combined efforts of phytopathologists and entomologists marks, therefore, a most important forward step. The recent hearings relative to the potato disease quarantine, under this act, have served not only to emphasize its importance, both commercially and educationally, but also to point out important new duties for plant pathologists. In order wisely to administer such quarantine measures there must be international cooperation among phytopathologists in determining the occurrence and seriousness of plant diseases. But while we are thus beginning to guard our borders against potato wart and other dangerous foreign diseases, what are we doing within our own territory? For example, we know that there is an alfalfa disease (*Urophlyctis alfalfae*) similar to the black wart of potato in its nature and destructive possibilities, as yet apparently limited in its distribution to a few western alfalfa-growing sections.[15] No official steps have as yet been taken, so far as I know, to make exact determinations of its present distribution or to guard against its being carried to other places on seed. This would seem to be a national rather than a state function and the national plant disease survey already referred to would seem to be the logical first step. In this connection the plan outlined by Orton for official inspection and certification as to health of seed potatoes is highly significant.[16] I believe it must commend itself for adoption with various other crops as well. There is no other place more important for guarding the health of crops than at the source of seed.

And finally, there is the question of disease resistance and immunity. Of course, the idea is not new; observations upon the relative liability of varieties to disease come to us from early times. But the clearer conception of the possibilities in this respect of plant improvement through breeding is recent. The relative success of the German and Scotch breeders in securing disease resisting potatoes is fully recognized.[17] The work started by Ward at Cambridge has raised our hopes relative to the possibilities of placing the studies of disease resistance on a scientific basis. The most stimulating results in America have dealt with resistance to soil fungi including Orton's work on cowpea, cotton and watermelon in the south and Bolley's on flax in Dakota. Such results as these and Norton's on asparagus rust resistance are to be regarded, not as final, but as merely suggestive of what I believe to be the most important future line of work in the control of plant disease, the breeding and selection of plants for local adaptation and disease resistance. If this is true then the fundamental problem deserving most serious consideration is, what constitutes disease-resistance? The difficulty of even defining the factors involved should not deter us from urging its importance and encouraging work upon it along all possible lines of attack.

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[15] O'Gara, P. J. *Urophlyctis alfalfae*, a fungus disease of alfalfa. Science, N. S. **36**: 487. 1912.

[16] Presented in a paper before the Annual Meeting Wisconsin Potato Growers' Association, Nov. 20, 1913. To be printed in the Proceedings of this Association, which may be had from J. G. Milward, Sec'y., Madison, Wis.

IX. Conclusion

In conclusion let us emphasize that, if progress in plant pathology is to continue as rapidly as we hope, those who are responsible for its direction should realize the limitations of the individual workman, and the necessity for division of the labors involved.

The demand today upon the American phytopathologist is almost equally urgent for four types of service: (1) college teaching, (2) extension teaching, (3) inspection, (4) research. In how far are these compatible?

The ideal college teacher must be an investigator, but until we have passed the present stage of rapid growth in our state colleges, nothing comparable to the proper proportions in the division of his energies between these fields is practicable. The duties of public adviser or extension service in plant pathology may not be wholly incompatible with college teaching or station research, although at times seriously distracting. I am, however, convinced that in such matters the professional plant pathologist may in general wisely delegate the responsibility to act as spokesman to his associates in horticulture and agronomy. The nature of a disease and its mode of control once settled, the application of control measures becomes simply one factor in the complex of cultural operations for the execution of which the above departments become responsible.

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[17] See Stuart, Wm. Disease resistance of potatoes. Vt. Agr. Exp. Sta. Bul. 122. 1906.

Plant disease surveys, inspection and quarantine service belong in still another class and deserve the attention of experts in plant pathology. But back of all these must stand the investigator, with time and faculties kept free for his fundamental work; for research is the most exacting of all taskmasters. While no one realizes more keenly than do I the present impracticability, in general, of restricting our responsibilities along any such clean cut lines, nevertheless I am convinced that it is only as we clearly define these ideals and approach more nearly their realization that we are to secure the best results. It is encouraging, therefore, that these responsibilities are being divided in an increasing number of state institutions and that the proposed reorganization in the United States Department of Agriculture follows similar lines, differentiating research at least from the other fields of work.

If in this overlong discussion I have taxed your patience by emphasizing more the problems than the progress in plant pathology, it has been with a two-fold purpose. On the one hand, I have hoped thus to win your continued charity toward the plant pathologist, in view of the complexity of the problems which he must meet, administratively as well as scientifically. On the other, I have wished to urge your continued cooperation along the two lines: first, in training young men for the profession,—the best training our botanical institutions can give, with increasing attention to physiology; and second, in sharing, in the future as in the past, in the responsibility for focusing attention upon the fundamental problems and fixing standards by which rightly to measure progress toward their solution.

University of Wisconsin.

SOME OBSERVATIONS ON THE ANATOMY AND OTHER FEATURES OF THE "BLACK KNOT"

ALBAN STEWART

WITH PLATES IX AND X

Some three years ago, while I was connected with the department of botany in the New Hampshire Agricultural College of Durham, New Hampshire, I became interested in the study of the "black knot" fungus, *Plowrightia morbosa* (Schw.) Sacc., which causes the black enlargements on the branches of plum and cherry trees, and from which character the fungus receives its common name. A considerable amount of material was collected from *Prunus virginiana* L. in that vicinity at intervals of two or three days, from the early part of May until July, and properly preserved for future study. I also made some cross inoculations on the cultivated plum, but as I was called elsewhere soon after this, I did not pursue this part of the work further. I began the study of my material in Professor Thaxter's laboratory at Harvard University about six months after I left Durham, but after sectioning it was found that the material was all too old when it was collected to show early stages of development of the knot, so I abandoned the work for the time being.

Upon coming to the University of Wisconsin in the autumn of 1911, I found that my colleague, Professor E. M. Gilbert, had already begun the study of this fungus, and as neither of us had progressed far at this time, we agreed to divide the work, Professor Gilbert to study the morphology of the fungus while I was to confine my work to the anatomy of the knot itself. The results I have obtained are given in the following pages.

Papers dealing with this fungus in a general way are very numerous, probably owing to its wide distribution in this country. Nearly every Agricultural Experiment Station, in the states where this fungus occurs, has dealt with it more or less with reference to methods of eradication and control. Papers, however, on the morphology of the fungus and of the knot itself are very few, owing possibly to the supposedly difficult technique involved in preparing proper sections for study.

About the first paper to appear which deals in any way with these points was that of Peck (6). In a communication read before the Albany Institute this author discusses in a general way, among other subjects, the external morphology of the knot, the structure of the conidia, the time of ripening of the ascopores, methods of control, etc. The next paper to appear on this subject was by Farlow (3), in which the life history of the fungus is described in detail. The histology of the knot is also discussed rather fully and the results given have been largely copied by all of the subsequent writers on this subject. It is evident from the figures that only later stages in the development of the knot were studied in the preparation of this paper. Crozier (1) speaks briefly of the structure and time of ripening of the ascospores. He also mentions the fact that when the knots are young the distinction between bark and wood still exists, the cambium line being deflected outward through the knot, a statement which seems to have been overlooked by subsequent writers. Humphrey (4) describes some interesting results obtained from germinating and growing spores on culture media, stages in the life history of the fungus, and some of the structural characters of the knot. Lodeman (5) offers suggestions as to how the fungus gains entrance to the tissues of the host, and deals with the disease in an economic way. So far as I have been able to learn the above cited papers are all that offer anything new concerning the anatomy of the knot or of the morphology of the fungus.

The choke cherry, *Prunus virginiana* L., grows very abundantly in the vicinity of Durham. Thickets of these bushes occur along roadsides and waste places which are usually badly infected with the black knot fungus. I found, however, that this was the only species of cherry that was infected in that vicinity as both the pin cherry, *P. pennsylvanica* L., and the wild black cherry, *P. serotina* Ehrh., grow there, but in no instance was either of these species found to be infected. In one place near Durham pin and choke cherries grow so close together that their branches intermingle, but in no instance were the branches of the pin cherry infected, although those of the choke cherry were covered with knots. Thickets of choke cherry bushes grow all around the agricultural college farm at Durham, but in pruning the orchard of cultivated plums, during the year that I was there, only two or three small knots were discovered upon the trees. The choke cherry and wild plum, *P. americana* Marsh., are infected in the vicinity of Madison, Wis., but no other species so far as was observed by the writer, although Professor Gilbert found an infection on the pin cherry in one instance. The wild black cherry grows here, sometimes in badly infected thickets of choke cherry bushes, but it is not infected. It is evident that there is either a great difference in the susceptibility of species of Prunus in different localities, or there is a different physiological form of fungus for each species.

Knots caused by this fungus arise from two kinds of infections[1]: primary, those which result from the infection of the host plant by spores, and which reach their full development the year following the infection, and secondary, those which result from the spreading of the fungus through the tissues of the host from knots already formed.

Knots arising from primary infections usually occur on young branches either of the current year's growth or on those not over two or three years old. They do, however, occur at times on much older branches, as instances were noticed where knots had been formed on branches a centimeter or more in diameter and which had six or seven rings of annual growth. If cross sections of young branches are examined, after they have begun to swell, it will usually be found that the xylem is greatly altered into, or nearly to, the pith at the distal end of the knot, while the proximal end will have quite a zone of normal xylem surrounding the pith. From this one would judge that most of the primary infections take place at about the time, or shortly before, the cambium begins its activity in the spring, which is about May 1 at Madison.

The external modifications following secondary infections begin to appear two or three weeks after the first conidia are formed. These are shown first by a cracking open of the bark and a slight swelling of the stem beyond the knot, which spreads farther as the season advances. The infection apparently extends beyond the swollen area at times but in such instances the extent of the disease can usually be determined by examining cross sections of suspected areas with a hand lens, since the tissue in such areas usually has a light brown color. Cross sections of both primary and secondary infections may show either a small or a large part of the circumference of the stem altered as a result. In either case, however, there is probably a progressive growth of the fungus around the stem as the season advances, resulting either in the girdling of the stem or the alteration of only a sector of it.

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[1] In this article the effect of the disease and not the cause is being considered. On this account the terms infection and infected will be used in a rather broad sense, and it is to be understood that the fungus was not always found in contact with the cells or tissues in question.

Abnormalities in the tissues of the stem caused by infection are usually deeper at the leaf gaps than in the internodes between, probably owing to the fact that more parenchyma is present in such areas than elsewhere. Lodeman (5) suggests that the fungus gains entrance to the stem at the axil of the leaf or about the bud situated in its axil. The great amount of disturbance might also be accounted for by this means if it were not for the fact that the leaf gaps in stems infected secondarily show quite as much disturbance as those infected primarily.

In young normal twigs of the choke cherry the rays are mostly uniseriate in the first ring of growth, with a few multiseriate rays two or three cells wide. In traumatic areas the multiseriate rays may be considerably broader in this ring, those having as many as seven cells in cross section having been noticed. Cross sections of six-year-old stems, such as is shown in fig. 1, show a proportional increase in the number of multiseriate rays and occasionally it can be seen that the formation of these may come about by the fusion of adjacent uniseriate rays. Tangential sections of the older wood, fig. 3, show that approximately the proportion of uniseriate to multiseriate rays is as two to one. Parenchyma is confined largely to the rays with an occasional cell in among the fibers and other elements of the wood, the small development of wood parenchyma being a character common to all of the Rosaceae, except the Tribe Chrysobalaneae, according to Solereder (7, p. 309). The vessels are small in size and are usually arranged in one or more zones in the early spring growth, beyond which they may become scattered so that it is often difficult to determine where the spring growth ceases and the summer growth begins. The tracheids in the normal wood are distinctly of the pitted type.

The stimulating effect of the fungus first makes itself manifest in the vicinity of the cambium in knots resulting from both primary and secondary infections, but the position and identity of the cambium as a whole is not so greatly changed as has been supposed. A portion of a cross section of a stem taken at Madison on April 6, approximately a year after infection, is shown in fig. 8. In this section the cambium is present opposite the projecting segments of xylem but it is not present opposite the broad rays. Figure 9 is a photograph of a similar section taken some two months later, after the knot has fully formed and has become surrounded by the hard carbonaceous layer containing the perithecia. In this section the cambium is seen as a somewhat broken line just outside the xylem wedges, which are formed at this stage, so that the prevailing idea that the cambium is broken up and scattered during the development of the knot is incorrect. Usually the cambium is first stimulated to produce a greater or lesser amount of parenchyma of a somewhat transitional nature. With the exception of the early spring growth, normal xylem always contains a large number of fibers which have the usual angular appearance, with thick walls, and small lumina when seen in a cross section. When the results of infection first begin to appear, there is a change in the character of the cells formed, and those which would probably have been fibers under normal conditions, lose their angular appearance somewhat, the walls become thinner, and the lumina correspondingly larger. The change may be but slight next to the normal xylem, but farther away it becomes greater and can be studied best by following out a radial row of cells from the normal into the abnormal part. The parenchymatous character of these cells is shown by the fact that they contain an abundance of starch, nuclei are often present in them, and the pitting in their walls is distinctly plain. A considerable zone of these cells may be produced, there may be but few of them, or, occasionally they may be entirely absent. Since cross sections of these cells have more the appearance of fibers next to normal xylem than they do farther away, it suggests that the stimulating effect of the fungus might have become effective in their vicinity while they were still in a plastic condition and that they were further altered by its influence. These transitional parenchyma cells react more strongly to safranin stain than do the fibers near by, so there can be no difficulty in determining whether they are present or not by this alone.

After the production of the transitional parenchyma, the cells formed are usually more distinctly parenchymatous in character. These cells are larger and their walls thin and unlignified. Occasional fibers or groups of fibers are often found scattered through them. This condition is usually more pronounced towards the center of the infected area, when seen in cross section, while towards the sides, the xylem may differ but little in appearance from that ordinarily formed except that it occurs in long radial segments separated from similar adjacent segments by the broad rays which are formed at this stage (see fig. 8). Parenchyma may continue to be formed until the end of the growing season, but usually small segments of summer wood are produced late in the season which appear as small islands, just inside the cambium, surrounded on all sides by parenchyma.

The presence of the fungus in the stem also stimulates the formation of an abnormal amount of ray tissue, shown to best advantage in areas where the remainder of the xylem has not been too greatly changed into parenchyma. A photograph of a tangential section of the normal wood of the choke cherry is shown in fig. 3, in which none of the multiseriate rays are over four cells wide. Fig. 6 is a photograph of a similar section from an infected stem, taken on February 22, in which the broad rays have become many cells wide, simulating the structure of compound rays. This section really appears more like one through the inner bark, but that it is through the xylem is shown under high magnification, because the rays are bordered by the usual xylem elements. Similar sections from specimens collected on April 29, at about the time the growth begins, show the same condition, but specimens collected some three weeks later, after the infected branches have become greatly swollen, show that these rays have become further altered by the growth of the knot during the second year of its development. A photograph of such a section is shown in fig. 4. Near the center of the figure are two small rays surrounded by tracheids and fibers, while to the right and left are bands of similar elements bordering adjacent rays. The small ray to the right of the center is one that under normal conditions probably would not have had great vertical depth. It has become so broad here that its tangential diameter is fully as great as its vertical. Instances were noticed where the tangential diameter was even greater than the vertical. A photograph of a similar section taken from a specimen collected on June 11, after the knot has reached its maximum size, is shown in fig. 5, in which the rays are still broader, and it will be noticed that some of the elements bordering them have been pushed fully 45° from their usual vertical position. There has also been a considerable increase in the size of the cells which constitute the rays as the magnifications in figs. 3, 4, 5, and 6 is the same.

The uniseriate rays are not altered as far as could be determined, or at least not to the same extent as are the multiseriate rays. Many uniseriate rays occur in the xylem of infected areas but the multiseriate rays are all much broader than they are in normal wood. It seems probable that when the stimulating effect of the fungus reaches a given area the cambium in that locality only produces an abnormal amount of ray tissue opposite the multiseriate rays.

From the above figures it can be seen that those portions of the infected xylem which are included in the knot are made up of a ray tissue to a considerable extent. Farlow (3, p. 446) evidently saw the ray-like character of the cells in the knot but did not recognize their full significance as he says: "In the knot we find bast fibers, wood cells, and dotted ducts; but the prevailing tissue consists of dotted, rectangular, parenchymatous cells, with very thick walls, which closely resemble the cells of the medullary rays."

At the beginning of the second season the growth of the knot is very rapid. The small segments of summer wood, formed just inside the cambium at the end of the previous seasons growth, are pushed outward by the growth of the parenchyma cells and the cells of the broad rays. This growth is due largely to an increase in size of both parenchyma and ray cells, but whether or not it might also be partly due to cell division was not definitely determined. None of these cells are in process of division in any of the sections examined, but a few instances were found in which a cell appeared to have two nuclei. Some of the nuclei were found to be slightly constricted at the center, a fact which suggests the possibility of amitosis in these particular cells.

The small segments of summer wood which were formed at the end of the previous season's growth, are pushed outward by the growth of the surrounding cells. The radial segments of xylem, such as are shown in fig. 8, are torn away from the rest of the xylem to which they were connected, and are pushed outward in a similar manner. One may often find instances where this is just beginning to take place and the gap of separation is but slight. These segments may become more or less broken up as they are pushed outward, so that fragments of them can be seen scattered around through the parenchyma of the knot. Their position is often such as to leave no doubt about their origin. In this pushing out of the radial segments of xylem and the isolated segments of summer wood, the cambium is also pushed outward, so it comes to lie much farther away from the center of the stem than before. After this has taken place it begins to form xylem of the current year opposite the xylem that has been pushed outward; the continuation of this activity results in the production of wedge-shaped masses of xylem. Between these still other wedges may be formed which are similar to those above mentioned except that they are not in contact with any of the xylem of the previous year's growth toward the center. The wedges are all well separated from each other by the rays, which have further broadened, so that cross sections through the infected part have a superficial resemblance to those of semi-herbaceous stems in which the fibrovascular bundles are separated from each other by broad rays. This is especially true in knots on young stems which have become changed throughout their entire circumference and into the pith. The structure of these wedges usually appears to be quite regular in cross section, but longitudinally they show a considerable amount of wood parenchyma and scalariform tracheids to be present. It is evident that these tracheids are truly scalariform and not apparently so, owing to tertiary thickening, because none of the ordinary bordered pits are present in their walls. These scalariform tracheids are also very common in the cortical portion of the knot at this stage, but are not present in normal adult wood.

Tangential sections through the wedges (see fig. 5) show that the elements that compose them are very much bent and twisted, a condition that is brought about by the broadening of the rays. Radially, the course of these elements is usually somewhat wavy.

The development of the wedges is quite uniform for a while but sooner or later they may become altered in appearance by the production of a considerable amount of wood parenchyma along their sides. At about the time that this is taking place, there is a sudden growth of the rays in a radial direction. In this growth the position of the cambium opposite the rays is very much disturbed and portions of it are pushed outward into the cortical portion of the knot and more or less torn apart. It is often the case that the segments of the cambium opposite the rays are simply broken in two by this process and the free ends thus formed are pushed out into the bark, but otherwise remain in contact with the cambium opposite the xylem wedges. In the misplacement and disorganization of the cambium opposite the broad rays, its cells may become turned over more or less at right angles, so that tracheids, vessels, and fibers are commonly to be found in this altered position. The cambium may become completely broken up in these misplaced segments, resulting in the production of isolated xylem cells, or groups of a few of them together, scattered around through the cortical portion of the knot. Sometimes a considerable number of cambium cells may remain together and develop the usual elements to which they give rise but in a very much altered position. A condition of this kind is shown in fig. 7. The outer ends of two adjacent xylem wedges are shown on the lower side of the figure, each of which is subtended by a triangular shaped mass of phloem cells. These wedges are separated from each other by a broad ray, opposite which there is an overturned segment of xylem extending straight out into the bark, with the long axes of its cells at right angles to their usual position. Uniseriate rays occur in this segment but the view presented of them is the one usually seen in tangential sections of the wood. A more highly magnified photograph of the outer portion of this segment is shown in fig. 10.

When the disorganization of the misplaced cambium segments has gone still further a condition similar to that shown in fig. 11 is commonly found. This photograph was taken from a radial section cut through the cortical portion of a mature knot, and shows a band of fibers and tracheids extending diagonally across the photograph, which assumes an almost vertical direction near the center of the figure. Fibers and tracheids maintain this direction for a short distance and then bend almost at right angles and extend outward nearly to the stromatic layer shown on the right of the figure. Many of these misplaced elements are scalariform tracheids similar to those above mentioned. Radial sections through the region of the cambium sometimes show that a portion of a xylem strand will be in its normal upright position for some distance, and then it will suddenly bend outward into the bark and become lost. Cambium cells usually accompany these, showing that such strands have been formed in the place that they occupy, and that they have not been formed in their normal position and then thrown out of place by the subsequent growth of the knot.

In referring to the structure of the knot Duggar (2) states, probably basing his statement on some previous article, "Bast fibers, parenchyma cells, and even vessels may be found in this heterogeneous mass in which all of the associations of cells normally present have disappeared. This abnormal condition is apparently brought about by the breaking up of the cambium and a resulting development of all the various cell forms to which it may give rise in the diverse isolated areas." This is the prevailing idea concerning the development of the knot, but that it is not entirely correct is shown by the descriptions that have been given. The cambium as a whole retains its normal position between the xylem and phloem, and forms a more or less broken cambium ring through all stages. It is only from the broken up segments of the cambium opposite the broad rays, that these misplaced elements arise.

The changes that take place in the bark, during the first season's growth after infection takes place, are not so marked as are the changes in the xylem. The rays broaden, continuous with the broad rays in the xylem, and they may become bent more or less. The bark may thicken to some extent but the thickening is seldom very pronounced. The phloem is evidently not greatly interfered with physiologically, because vigorous shoots may continue to develop from the knot and from beyond it, during both the first and second seasons' growth after infection becomes evident. This may happen even on stems that have become completely girdled. Where parenchymatization has been very pronounced in the xylem, and when isolated segments of summer wood are not produced during the first season's development of the knot, the cambium may disappear and there will be no line of separation between wood and bark. In such cases, however, the cambium is reorganized at the beginning of the following season because xylem wedges are always formed across such areas.

The changes that take place in the bark during the second season's development of the knot are probably as great as those that take place in the xylem. Owing to the extension of the rays from the xylem into the bark, the breaking up and disorganization of the segments of the cambium opposite the rays, and the subsequent development of these cambium cells into their various elements, it is difficult to determine what part of the tissue in this portion of the knot arises from cells normally present, and what part from cells that have become pushed into it. A considerable amount of phloem is produced, usually in triangular shaped masses opposite the xylem wedges (see fig. 7). The outer part of the bark is not materially altered. A short time before the conidia appear in the spring, longitudinal cracks are formed in the cork, over such areas, disclosing the greenish yellow tissue below. Cross sections at a little later stage than this reveal the condition shown in fig. 12, in which all of the outer part of the bark, including the bast fibers, is being sloughed off. This is apparently brought about by the formation of fan-shaped masses of hyphae just inside the bast fibers. These masses unite later, one with the other, and form a thick stroma around the outside of the knot. Farlow (3) states that "The bast fibers are less altered in their direction and appearance than the other elements of the stem." This is due to the fact that they are located in a region that is not much changed in structure, so that they retain their normal position until they are sloughed off with the rest of the outer bark. The prevalent idea that bast fibers are included in the mature knot is probably not correct.

A cross section of a four-year-old stem infected with a knot is shown under low magnification in fig. 9. This knot has reached its maximum size and is covered on the outside with the black stroma containing the perithecia. As it illustrates well the structure of a mature knot it seems best to give it some description. The greatest diameter of this knot is 11.5 mm., of which 7.5 mm. is composed of xylem and the remainder of bark. The stem just below the knot has a diameter of 3 mm., of which 2 mm. is xylem, so that in this instance at least the proportional increase in thickness of both wood and bark had been about the same. About three-fourths of the circumference of the third ring of growth is changed in its outer part, and shows that the stimulating effect of the fungus reached this particular portion of the stem somewhat late in the growing season. Just above the normal xylem, near the center of the figure, the transitional parenchyma forms a dark band separating the xylem from the true parenchyma above. The parenchyma forms a light colored crescent-shaped mass above the center of the figure with dark colored radial bands of hyphae, and bits of last year's xylem scattered through it. An arch of xylem wedges borders the parenchyma above and becomes connected with the normal xylem on the right side of the figure while on the left side the conditions are further complicated by the fact that a lateral branch is given off at the point where the section was taken. The xylem on the lower side of the figure has not become greatly changed except that some of the multiseriate rays have become very broad in the outer ring of growth. It should be noticed that while this ring has not been greatly altered structurally on this side of the stem, its breadth has become greater than the combined breadth of the three preceding rings of growth just inside of it. The cortex opposite this portion is not altered. It is interesting to note that while strands of hyphae can easily be seen on the opposite side of the knot, even under a very low magnification, apparently none are present in either the wood or bark on this side. A broken cambium ring, made up of dark colored segments opposite the xylem wedges, can be seen to be continuous with the cambium opposite the xylem on the lower side of the figure. Farlow (3, p. 445) says: "If in midsummer or winter we make a cross section of a knot more than a year old we shall find one more layer of wood on the sound side of the stem than on the side of the knot. In other words, on one side, the formative power of the cambium has expended itself in forming a new layer of wood and bark (phloem), and, on the other, irritated by the presence of the fungus, it produces a mass, the knot, in which all distinction between wood and bark has been lost." A large number of knots have been examined during the second season of their development, but so far none have appeared which do not reveal a condition similar to that shown in fig. 9. This section shows the same number of rings of growth on both sides of the stem, the outer ring being represented by the xylem wedges on the infected side. A condition similar to this will always be found unless infection took place near the beginning of cambial activity the year before. In such cases the second ring of growth inside the cambium may not be seen because it is usually transformed into parenchyma, or if xylem was formed it has been pushed out of place by the growth of the knot as has been described. Cross sections of knots a year older than the one shown in fig. 9, will still show the xylem wedges if the interior of the knot has not been too badly destroyed by insects.

Small triangular-shaped masses of phloem are present opposite the most of the xylem wedges but the magnification in fig. 9 is too low to show them. They can be seen however in fig. 7. The dark zone around the outside of the section is the stroma containing the perithecia.

In stems younger than the one shown in fig. 9, a large cavity is sometimes formed in the center as the knot develops. In such cases it seems evident that a pulling force has been exerted and that the thin ring of xylem around the pith has not been strong enough to resist it, so it becomes pulled apart and may become more or less disorganized. In some instances it was found that the cells surrounding these cavities had begun to divide and had sent short filaments of cells into the cavity.

SUMMARY

1. Knots arise primarily from the infection of the stem by spores, and secondarily by the spreading of the fungus through the tissues of the stem from a knot already formed.

2. The changes induced in young stems of the current year's growth, as a result of primary infections, are usually apparent into or nearly to the pith at the distal end of the infection, while at the proximal there is a zone of sound wood surrounding the pith.

3. The vicinity of the leaf gaps is the seat of unusual disturbance in knots whether resulting from primary or secondary infections, owing to the large amount of parenchyma present in such areas.

4. Normal wood of the choke cherry contains uniseriate rays, and multiseriate rays two to four cells wide, the last of which may become further broadened as a result of injury to the stem.

5. As a result of the stimulating action of the fungus the multiseriate rays still further broaden in infected stems, simulating the structure of compound rays.

6. The production of the usual xylem elements is greatly inhibited during the first season's growth following infection, but there is a correspondingly greater production of parenchyma in the xylem.

7. The parenchyma cells formed in the xylem as a result of such stimulation greatly increase in size the year following their formation, and the rapid growth of the knot at this time is due largely to this fact.

8. There is an increase in the rate of division of the cambium cells on the side of the stem in which invasion occurs, and there may also be an increase in the rate of division on the opposite side of the stem when the fungus is apparently absent from its tissues on this side.

9. The cambium retains its normal position between xylem and phloem throughout the development of the knot, except opposite the broad rays.

10. Wedge-shaped masses of xylem are produced by the cambium during the second season's growth of the knot.

11. Isolated xylem elements, or groups of such, in the cortical portion of the knot, are formed entirely from the misplaced and

broken up segments of the cambium opposite the broad rays, and not from the breaking up of the entire cambium.

12. Scalariform tracheids are formed by many of the misplaced cambium cells in the cortical portion of the knot. Only pitted tracheids occur in normal adult wood.

13. The proportional increase in the thickness of both wood and bark is about the same in the development of the knot.

14. The outer portion of the bark is not altered materially and is sloughed off just before the conidia appear.

METHODS

In preparing sections for study and photomicrographing it was found, after considerable experimenting, that the best results could be obtained by cutting the knots without imbedding. Sections 10μ in thickness could be easily obtained in this way which were thin enough for what was required. Where softer portions alone were to be studied the usual paraffin method was employed. Iron-alum haematoxylin and safranin were used by the usual method employed in staining wood sections, except that after removing the excess of safranin in 95 per cent alcohol the sections were transferred for a short time to acid alcohol and then to weak ammonia water. Without this, good differentiation was not obtained.

The writer wishes to express his thanks to Professors Farlow and Thaxter for the many courtesies shown him while he was working in their laboratories at Harvard University. He wishes also to thank most heartily Professor L. R. Jones, of the department of plant pathology, and Professor C. E. Allen, of the department of botany, University of Wisconsin, for much advice and assistance.

The negatives for figs. 2 and 10 were made by Mr. M. E. Diemer of the Forest Products Laboratory.

DEPARTMENT OF BOTANY,
UNIVERSITY OF WISCONSIN,
MADISON.

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DESCRIPTION OF FIGURES IN PLATES IX AND X

All figures are of the choke cherry, *Prunus virginiana* L.

Plate IX

Fig. 1. Cross section of a portion of a normal stem. × 44.

Fig. 2. Cross section of a portion of an infected stem at junction of uninfected and infected xylem. × 100.

Fig. 3. Tangential section of normal wood. × 44.

Fig. 4. Tangential section of a portion of a knot taken May 15, showing broadening of the rays. × 44.

Fig. 5. Tangential section of a portion of a knot taken June 11, showing still further broadening of the rays. × 44.

Fig. 6. Tangential section of infected xylem taken February 22, showing an early stage in the broadening of the rays. × 44.

Plate X

Fig. 7. Cross section of a portion of a mature knot, showing the outer ends of two xylem wedges, a broad ray between, and overturned xylem elements in the bark opposite the ray. × 44.

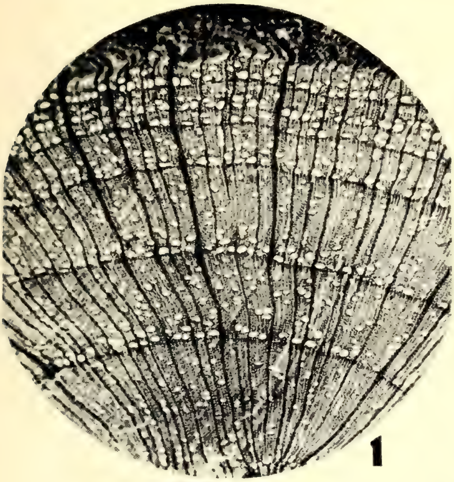
Fig. 8. Cross section of a portion of an infected stem taken April 6, showing broad rays and parenchyma in the xylem. × 44.

Fig. 9. Cross section of a mature knot. × about 5.

Fig. 10. A more highly magnified portion of Fig. 7, showing uniseriate rays in overturned xylem segment. × 100.

Fig. 11. Radial section through a mature knot showing the general direction of some of the misplaced xylem elements. × 44.

Fig. 12. Cross section of the outer portion of a knot, in an early stage, showing the removal of the outer part of the bark. × 44.



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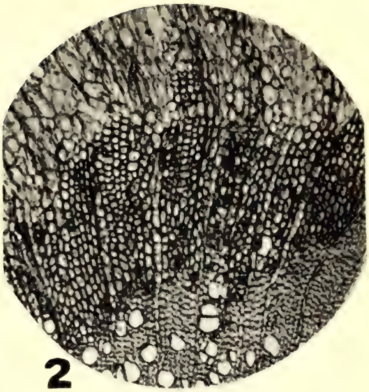

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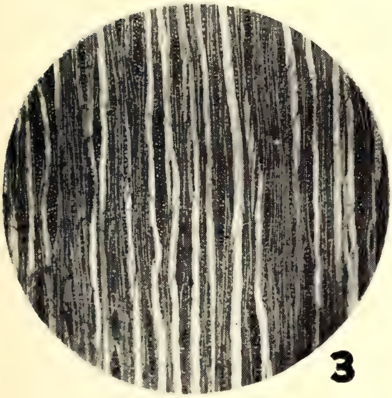

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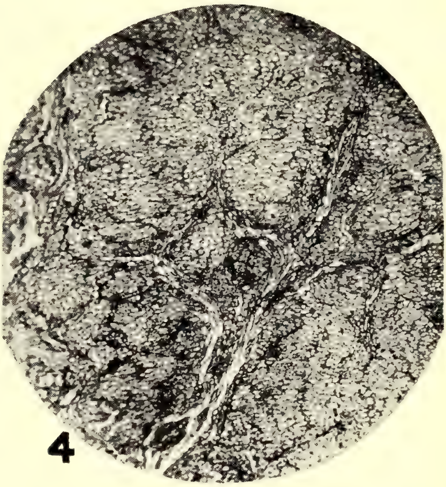

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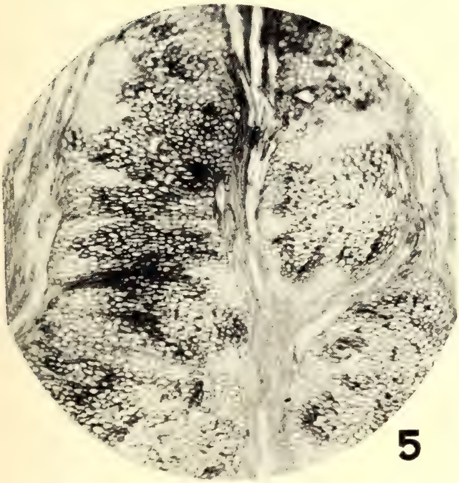

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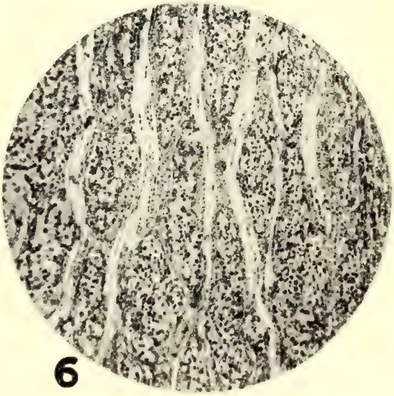

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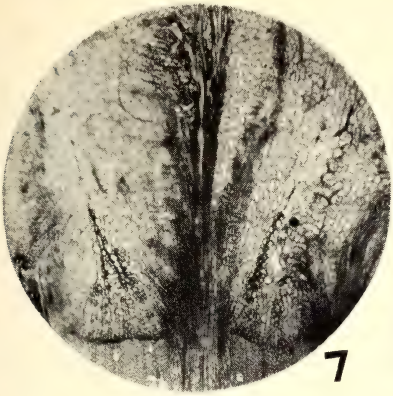
STEWART: OBSERVATIONS ON BLACK KNOT.



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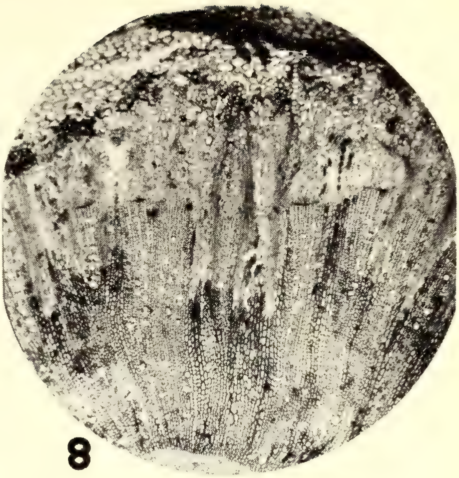

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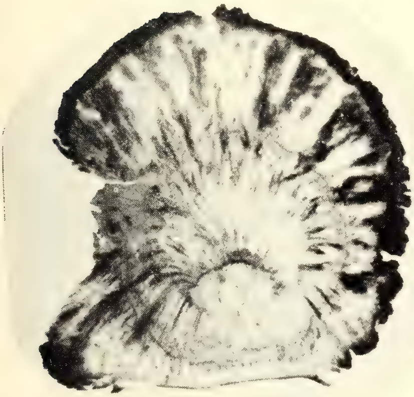

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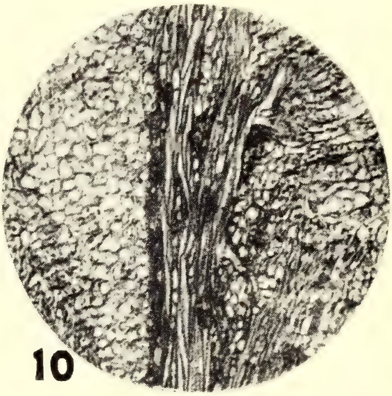

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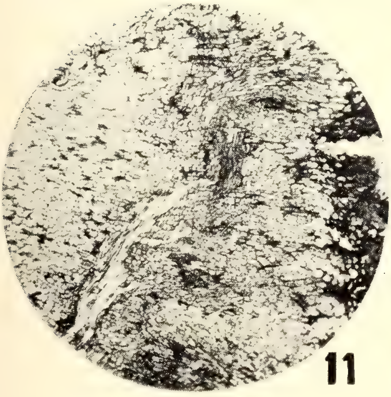

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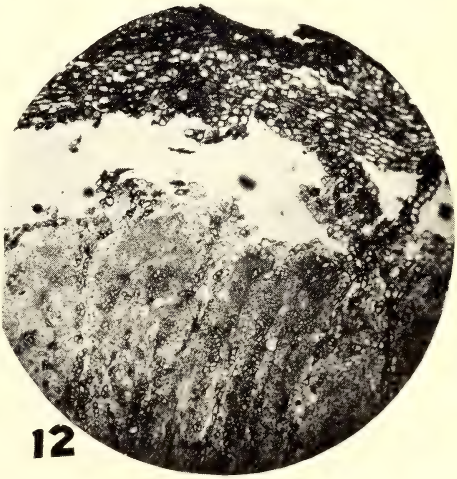

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STEWART: OBSERVATIONS ON BLACK KNOT.

CLEAVAGE IN DIDYMIUM MELANOSPERMUM (PERS) MACBR.

R. A. HARPER

WITH PLATES XI AND XII

Many recent papers have confirmed the contention that cell division in the sporanges of algae and fungi is a process of progressive cleavage by surface furrows as against the older conception of simultaneous division by cell plate formation. Still the unique type of cell division by repulsion of the coarser cell inclusions and their heaping up in neutral planes which delimit the oospheres, described by Farmer and Williams (11) for the oogones of Fucus still remains unquestioned and little has been added to our knowledge of the mechanics of the cleavage process. The older authors were for the most part dominated in their descriptions of spore formation in sporanges, first by the erroneous theory of cell formation put forth by Schleiden, and later by the conceptions of cell plate formation derived from studies on the higher plants. This older literature has been several times adequately summarized and need not be referred to further here (15, 29).

Timberlake (31) describes the swarm spore formation in Hydrodictyon as taking place by means of furrows which cut in from both the plasma membrane and the tonoplastic surface of the primordial utricle. Klebs's (18) figures also certainly suggest that the division is a progressive process.

Klebahn (17) speaks of the division of the oogonia in Sphaeroplea as a cleavage and his figure (no. 2) shows furrows cutting up the multinucleated oogone into the eggs. He does not describe the process of division in the antheridia.

Swingle (29) has proved beyond question that in Rhizopus and in Phycomyces the cell division consists in a progressive cleavage by which the multinucleated spore-plasm is cut up into the definitive spores. In Rhizopus the furrows originate primarily from the periphery; in Phycomyces they originate largely from vacuoles in the spore-plasm. The process in these forms is similar to that in the sporanges of Sporodinia and Pilobolus (16) but with striking and characteristic differences.

Conard (6) finds the process of spore formation in *Lycogala exiguum* entirely similar to that I have described for Fuligo (14). Rytz (28) finds that in *Synchytrium succisae* nuclear division continues during cleavage as I have described for *Synchytrium decipiens* and for *Fuligo varians* and describes the cleavage process as essentially similar to that in *S. decipiens.* Rytz's figures establish the existence of surface furrowing beyond question for this form.

Bally (2) is inclined to accept the conception of cleavage by surface furrows rather than by cell plate formation for *Synchytrium taraxaci,* though stating that his material did not show the stages necessary finally to settle the question. Tobler (33) believes that cleavage takes place by surface furrows in *S. pilificum* and *S. pyriforme.*

A number of other recent authors, supposedly using modern methods of technique, still report cases of so-called simultaneous cell division of multinucleated cell bodies. Baum (5) claims that in Chlamydomucor and Coprinus and a number of other forms simultaneous formation of a cell plate across the entire hypha may occur, while admitting that in hyphae with a central vacuole the cell plate is regularly formed as a peripheral ring which progressively widens inward. He admits further that this same progressive formation of a cell wall may occur in hyphae which are filled with dense cytoplasm through their entire cross section. Baum holds that the essential feature in the process in both cases is the accumulation of granular material to form a cell plate, which is then transformed into a wall and in this he follows the older views of Strasburger. These granules he identifies with the so-called cellulin granules of Pringsheim. Baum's evidence for the existence of simultaneous formation of a cell plate across the entire diameter of a hypha is quite inadequate. His figure 1, *Tafel III,* probably represents a stage after the cell division is complete.

Davis (7) describes the formation of zoospores in *Saprolegnia mixta* as a process of cleavage from the central vacuole outward and accepts the account of earlier authors as to the loss of turgor of the sporange when cleavage is complete and as to the subsequent apparent fusion of the newly delimited spores. His figures 33 and 34 are, however, quite unlike any cleavage process described elsewhere and he asserts that the final step in the separation of the spores consists in the breaking down of fine strands of cytoplasm by which the spores have been connected. For Derbesia, Davis (8) describes and figures the cleavage in the sporange as taking place by branching furrows from the periphery inward.

Löwenthal (20) describes zoospore formation in Zygorhynchus as simply the bounding off of a portion of protoplasm about each nucleus; but his figures show no stages in the process nor evidence as to how it takes place.

Kusano (19) claims to be able to distinguish two types of cell division in *Synchytrium puerariae.* The first type is a progressive cleavage by broad furrows leading to the formation of multinucleated sporanges. In the second type the sporanges are formed "by the precipitation of partitions in the compact cytoplasmic mass." Kusano's figure 6 probably represents young sporanges after cleavage is completed and growth has begun though he argues against this interpretation. It is an old and familiar observation which Wager (34) re-emphasizes that after cleavage is complete the spores may swell and grow so as to press upon each other to the extent of making the lines which mark their boundaries very inconspicuous, especially in living material. It is this condition which has apparently deceived Kusano. Kusano further erroneously identifies his second type of division with that described by Timberlake for Hydrodictyon. As noted above, however, Timberlake describes the cell division in Hydrodictyon as taking place by furrowing and as progressive. For Rhodochytrium, Griggs (13) says, like Kusano, that he observed both progressive cleavage by surface furrows and simultaneous segmentation and adds that the former was infrequent.

Barrett (4) describes the segmentation of the protoplasm in the sporanges of Blastocladia as proceeding from the periphery inward, much as I have described for *Synchytrium decipiens*, but he adds that the lines of division are first recognized as rows of granules which are at first more or less indefinite but which become more and more apparent till they are seen to outline the spores. There can be little doubt, however, as his figures 30 and 31 suggest, that what he describes as rows of granules are in reality cleavage furrows. For the sporanges of Olpidiopsis, Barrett (3) speaks of the division of the protoplasm as involving the formation of spore centers and states that as far as he could determine the fragmentation was simultaneous throughout the sporange much as described by Dangeard for *Synchytrium taraxaci.* He seems, however, in this case not to have really found the cleavage stages; for his figure 39 certainly represents a condition after cleavage is complete, and his figure 38, as he states, represents a stage in which segmentation has not yet begun. It is interesting to note that Bally

(2) is inclined to discard the erroneous description by Dangeard which makes the cleavage in *Synchytrium taraxaci* simultaneous while Barrett holds Dangeard's account is true also for Olpidiopsis.

Wager (34) notes again that the lines of demarcation of the spore-origins, in the cysts of Polyphagus, appear, disappear and reappear again as the older authors have observed in the sporanges of the Saprolegniaceae. He describes spore formation as due to cleavage from the center outward. When the clefts reach the plasma membrane the sporange contracts and perhaps the spores also swell.

Moreau (23) describes the spore formation in *Circinella conica* as beginning with an extreme vacuolization of the protoplasm. The vacuoles become irregular in form and break the spore-plasm up into fragments. The fragments are irregular amoeboid bodies and remain for some time connected by strands which finally break through. Just how this process is related to the cleavage by vacuoles as seen in Pilobolus and Phycomyces is not clear and Moreau's figures give little idea as to just how the vacuolization results in the fragmentation of the protoplasm. Moreau finds the cleavage in Rhizopus and Phycomyces to be essentially as described by Swingle and holds that, as in Circinella, contraction phenomena are an essential phase of the process. In *Mucor spinescens* Moreau finds that vacuolization of the spore-plasm results in the formation of long strands, with the nuclei in a single series. These strands become nodular or catenoid and break up into uninucleated or sometimes several nucleated spores.

For the Conjugatae and Desmids there is general agreement that as in Cladophora the cell divides by a circular cleavage furrow with simultaneous wall formation. Lutman (21) has described the process in detail for Closterium.

The conditions in algae such as Dictyota and Sphacelaria need further study. Swingle (30) and Mottier (24) describe a peculiar sort of plate formation without the presence of a central spindle. Tuttle (32) describes nuclear division in Oedogonium but says nothing definite as to the method of cell division.

McAllister (22) has recently described a cell plate formation in Tetraspora which is quite like that of the higher plants. The interesting possibility is thus suggested that as McAllister argues those algae which are in the line of ascent to the higher plants may have quite a different method of cell division than that found in Cladophora, Hydrodictyon and other well known types. Whether or not this conception is confirmed there can be no question that, in the best known forms of algal and fungous sporanges in which a multinucleated spore-plasm is cut up into one or relatively few nucleated spores, there is no trace of anything like the cell plate formed in the central spindle of the higher plants.

It is evident that there is as yet no final agreement as to the types of cell division characteristic of the different families of plants nor as to the substantial uniformity of the process even within such a genus as Synchytrium or such a family as that of the Mucorineae. Much more careful descriptive work is needed before the limits of variation and the features common to all types of cell division can be determined. The slime moulds are certainly favorable material for such studies and the comparison of cell division in spore sacs which are so similar in externals as are those of Didymium and the Mucorineae gives an excellent opportunity for determining the essential physical and mechanical features of the process independent of resemblances due to close relationship.

The cleavage process has not so far been described for any of the Myxomycetes with simple spore sacs. The sporange of Didymium with its dome-shaped spore mass, large columella cavity and characteristic columnar stalk imitates a mucor closely though the method of its formation is quite different and the parts cannot be considered as homologous. It is questionable whether the term sporange should be used for such diverse types. De Bary (9) introduced the usage substituting the term sporange for the still more objectionable term peridium as used by the older authors on the assumed resemblances of Myxomycetes and Gasteromycetes. It is, however, hardly worth while in the present state of our knowledge to attempt to give any definite morphological significance to such a term used as it is indiscriminately for organs of ferns, algae and fungi without regard to phylogenetic connections.

While the general shape of the dividing mass is strikingly similar in the sporanges of Didymium and the black moulds, in Didymium the spore-plasm at the time of cleavage is already pierced radially at regular intervals by the threads of the capillitium, while in the moulds it is practically a homogeneous mass. It is possible also in Didymium to obtain a view of the whole section of the dividing mass in a fashion not possible in the case of Fuligo and thus to study the mechanical changes in such a multinucleated cell regarded as a unit.

I have photographed the principal stages for the purpose of bringing out the essential features of the cleavage process free from all possible bias as to the distribution of the nuclei, the angles which the cleavage furrows make with each other, the outlines of the whole mass and the general progress of the process from the periphery to the interior of the spore-plasm. The material was collected near Madison, Wis., fixed in Flemming's weaker solution and stained in most cases with the triple stain though certain of the photographs are from iron haematoxylin preparations.

At a stage when the sporange has reached its full size (fig. 1) and is still milk-white in color the spore-plasm is dense and finely granular in the peripheral region and in the region immediately adjacent to the columella. The middle zone of the sporange on the other hand is still very vacuolar and almost foamy. This represents a condition in which the condensation of the protoplasm in preparation for the formation of spores is complete, except in the interior of the mass. The persistence at this stage of numerous vacuoles in this central region perhaps indicates that a gradual reduction of the cell sap has already been taking place during the early development of the sporange. If the sap passes off by evaporation the vacuoles will persist longer in the central region of the protoplasm.

The capillitium is already formed before the condensation of the protoplasm has been completely accomplished (fig. 2). It consists in Didymium of fine, smooth threads which pass radially outward from the central, dome-shaped columellar cavity to the peridium. The capillitial threads are attached at each of their ends, and we have thus in some respects a condition similar to that in Stemonitis where the capillitium consists of threads branching from a central stalk-like columella and ending peripherally in a very delicate network beneath the peridium. In Stemonitis the radial branches of the capillitium subdivide very freely; in Didymium, on the other hand, the capillitial strands branch scarcely at all. When they do divide the branches form a very acute angle with each other and run on almost parallel toward the periphery. As a result it is not difficult to find in sections lying in the proper plane some fibers that run from the center to the periphery in the plane of the thin microtome section. When mature the capillitial threads are smooth and polished and dark colored. In these early stages the protoplasm is in most intimate contact with the surface of the capillitial threads throughout (figs. 1 and 2). There is no tendency to the formation of vacuoles about the threads nor for the protoplasm to shrink away from them in fixation. At their inner and outer ends the threads swell slightly and become hollow where they join the peridium and columella respectively. Throughout the rest of their extent they are solid and show no surface sculpturing nor markings of any kind.

At this stage the nuclei are scattered rather uniformly through the protoplasmic mass (figs. 1 and 2). With the magnification used for figure 2 the nuclei appear as hardly more than dots and those less deeply stained are scarcely visible. In some cases there is a tendency for the nuclei to be surrounded with a vacuole. This, however, is true of relatively few of the nuclei in any particular section, while the remainder show themselves in close contact with the cytoplasm over their whole surface. Such cases can hardly be due to shrinkage in fixation, since they are scattered among other nuclei which show no such peculiarity. Nuclei enclosed in vacuoles have been observed in the root hairs of Chara and Debski (10) has shown that in this case the vacuoles do arise during fixation.

The nuclei at this stage are of very unequal size. The smallest nuclei are extremely dense chromatic masses. In some of these it is almost impossible to make out a nucleole; in others, however, and especially in those that are a little larger, by the use of the triple stain a red nucleole can be conspicuously differentiated from blue chromatin as in figure 7. I have not found any nuclear division figures in my sections at this earliest stage, though they are frequent at a very little later stage. We may distinguish three distinct types of nuclei at this stage on the basis of their size and appearance. The type of smallest size is that just described. Those of middle size are abundant and very typically differentiated. The nucleole is sharply defined from the chromatin, is globular in form and dense but transparent and stains bright red. The chromatin consists of strands and granules forming the ordinary netted or reticulated figure of resting nuclei. The nuclear membrane is sharply defined and the nuclear cavity contains a considerable amount of clear cell sap in the meshes of the chromatin network. Nuclei of the third type are distinctly larger, and show very different staining qualities than the smaller nuclei. Their general consistency is very like that of the cytoplasm and they do not appear nearly as conspicuous as do the other two types. Their nuclear membranes are, however, very sharply defined. Their nucleoles also are bright red stained globules no larger than the nucleoles of the previous type, although the nucleus itself may have a diameter $\frac{1}{3}$ to $\frac{1}{2}$ greater. The chromatin shows very little affinity for ordinary chromatin stains and appears almost homogeneous throughout the nuclear cavity. There is no sharp distinction of chromatic strands and nuclear sap to be made out. The chromatin is apparently distributed in the form of fine granules throughout the nuclear cavity. The condition of these nuclei resembles somewhat that found in the nuclei of the onion root tip at some distance back from the apex where they are not dividing rapidly and pass over into a resting condition.

The homogeneous dense stage in the development of the sporange is followed immediately by a condition which apparently represents a further contraction and loss of cell sap on the part of the protoplasm. At this stage we find abundant vacuoles, or what appear at first glance as vacuoles, arising in the peripheral region of the spore sack (figs. 3, 4, 5). These figures, as is also the case with figures 2, 6–10, 15 and 17, represent sections cut more or less horizontally through the developing spore sack, the columella cavity appearing as a more or less circular opening in the center. A little closer examination shows that each of these apparent vacuoles is pierced by a capillitial thread which runs straight through its central axis or may be slightly curved (figs. 9, 15, 17). These apparent vacuoles are in reality cavities formed by the withdrawal of the protoplasm from the surface of the capillitial threads at particular points. It is plain that the protoplasm is bounded off against the surface of the capillitial thread by an osmotic membrane, and that it has exuded liquid through this membrane into a space formed about the surface of the thread. The increase in amount of this exuded liquid leads to the formation of an almost globular droplet, which on superficial observation has all the appearance of an intra-cytoplasmic vacuole. Several of these droplets may be formed in a series not very far apart on the same capillitial thread. The row of watery droplets is in a sense suspended on the capillitial thread. It is to be seen at once that these droplets are in the strictest sense exterior to the protoplasmic mass, since the membrane of the apparent vacuole which encloses them is in reality continuous along the capillitial thread with the external plasma membrane of the whole sporange. The droplets are not of course perfectly spherical, but form more or less extensive catenoid-like series on the capillitial threads. Their formation seems to consist, as noted, in a further extrusion of what we may call cell sap from the protoplasm, the exudation now taking place along the surface of the capillitial threads as well as upon the external surface of the protoplasmic mass as a whole. These sap cavities on the capillitial threads become very abundant, extending throughout the whole peripheral region of the spore-plasm. They next become connected along the threads and we have thus as it were canals running radially in the peripheral region of the cytoplasm and filled with a watery extruded liquid. Figure 5 shows the apparent vacuoles somewhat ellipsoidal in form as they run together. This change in their form and their resulting confluence are no doubt due to the continued extrusion of water from the protoplasm in preparation for spore formation. These watery sheaths open to the exterior at the ends of the threads, forming, as noted, tubular canals about the capillitial threads leading down into the protoplasmic mass. The extruded cell sap now apparently flows out quite freely, leaving the spore-plasm once more quite dense, but it does not again come into close connection with the surfaces of the capillitial threads (figs. 6 and 8). Such radial sections at this stage show thus what appear to be clefts running through the spore-plasm. That they are not furrows but are merely tubular depressions enclosing the capillitial threads is clearly shown in tangential sections of this same stage (fig. 10).

The contraction of the protoplasm and extrusion of liquid occurs also in the region next the columella so that we have the same appearance of clefts here (figs. 6, 8), and as the contraction continues it is possible to trace these tubular openings about the capillitial threads from the periphery to the center as continuous openings through the protoplasm (fig. 9). The appearance of these radial clefts sharply differentiates this stage from the earlier conditions shown in figure 2. As noted, a tangential section at this stage (fig. 10) shows the protoplasm pierced by rounded or angular openings in each of which the cross section of a capillitial thread is found. The angular openings represent the later stage and are formed from the rounded openings by the formation of cleavage furrows from their surface. These furrows cut into the spore-plasm and tend to divide it up into radially placed prisms or cylinders. Sometimes more than one capillitial thread will be found in a single opening. Very soon the furrows from adjacent openings come together making irregular branching series of clefts. More or less horizontal radial sections at the stage when the furrows first begin to form about the capillitial cavities are shown in figures 9 and 11. We see in such sections that the end of each prism or cylinder of spore-plasm tends to be rounded off so that the clefts are wider at the surface than they are deeper down in the sporange.

The furrows which have originated from the capillitial openings curve and branch in the peripheral region in such a fashion that they come to lie in a plane more or less tangential to the surface of the sporange and in this way begin to cut off blocks from the ends of the cylinders of spore-plasm formed in the initial cleavage stages. These tangential furrows begin to be formed before the radially placed cylinders and prisms have been entirely separated from each other. The cleavage is in reality a progressive process working from the surface of the spore sack inward, both by radially and by more or less tangentially placed furrows. An early appearance of these tangential furrows is shown in figure 9, and a slightly later stage in which the first peripheral blocks are almost or entirely cut off is shown in figure 11 at *a* and *b*.

These same stages in the cleavage process can be observed also in the inner layer of the spore-plasm next to the columella cavity (fig. 11). But in this inner layer the cleavage is always a little less advanced than in the outer layer. The whole process parallels apparently a continuation of the extrusion of cell sap which began at an earlier stage in the formation of the sap cavities around the capillitial threads (figs. 3 and 4), and the extrusion of sap is apparently most rapid where evaporation can go on most rapidly. We cannot, of course, regard the process of cutting up the spore-plasm as a mere matter of the drying out of the protoplasm due to evaporation. The clefts and furrows may be more or less filled with liquid at all times, and there is thus only a minor difference possible between the superficial and the central portions of the spore-plasm. Still, loss of water by way of the columella and stipe, which is filled with coarse concretions (see fig. 18), is doubtless slower than from the outer surface of the sporange and we find that cleavage is also slower next the columella than on the peripheral surface of the spore-plasm. There seems to be an obvious parallel between facility for water loss and rate of cleavage, but this in no wise excludes the possibility that chemical changes in the plasma membrane also favor the active extrusion of moisture through all these cleavage stages.

At a stage when the shrinkage about the capillitial threads has led to the appearance of the radial clefts shown in figures 6–10, the nuclei show quite commonly a tendency to be arranged in series adjacent to the shrinking protoplasmic surfaces (fig. 7). In sections this arrangement of the nuclei leads to their apparent distribution in rows along the radial clefts. This appearance in section, of course, is due to the fact that the nuclei really tend to occupy a layer of the cytoplasm next adjacent to the plasma membrane bounding the capillitial cavities. The majority of the nuclei appear in such rows, although a considerable percentage are irregularly distributed. In radial sections a row of the sort described is frequently to be seen in the protoplasm with no apparent capillitial opening near it. An examination of the next adjacent sections, however, will always show that these rows are either immediately above or below a capillitial thread.

The interesting fact is to be further noted that at the stage when the nuclei tend to show this characteristic arrangement along the cleavage surfaces a very high percentage of them are in the equatorial plate stage of division. This is especially true of the nuclei that have this characteristic position. Probably 75 per cent. of the nuclei at this stage that are arranged along the cleavage surfaces are in this stage of division. In figure 7 two of the nuclei in the equatorial plate stage are shown in polar view. The three resting nuclei may be distinguished by the presence of their nucleoles. Deeper in the protoplasm the nuclei may or may not be dividing. The further fact may be noted that the long axis of the spindle is quite commonly more or less parallel to the surface of the protoplasmic mass adjacent to which it lies. This is true only of the nuclei lying near the plasma membrane; those deeper in the protoplasm show no such orientation of their spindles.

It is interesting to note that a very considerable portion of the nuclei in Didymium at this stage are in a resting condition and that these resting nuclei are scattered irregularly amongst those which are dividing. The conditions in this respect are in sharp contrast with those in Fuligo where, during the cleavage stages, in any particular region of the aethalial mass, all the nuclei will be found in division at one stage or another. It is also in contrast with the conditions in Enteridium and Lycogala in which practically all the nuclei in a particular spore sack divide simultaneously. Series of spore sacks adjacent to each other may be found in which every nucleus is in the equatorial plate stage of division.

In Didymium it seems doubtful whether all the nuclei undergo division during the cleavage stages, though it is of course possible that the nuclei which are found in the resting condition at any particular stage may divide at a little later period. Since, however, as indicated above, the process of cleavage is rather rapid, it is quite possible in Didymium that only one nuclear division occurs between the time when the sporanges have reached their full size and the stage of complete ripening of the spores.

As noted, the cleavage process is initiated both from the outer and inner surfaces of the spore-plasm. A stage when almost an entire series of peripheral blocks have been cut off is shown in figure 12. At this stage the cleavage next to the columella is markedly less advanced. In the segments which have been cut off the nuclei are found to be irregularly distributed in their interior. There is apparently no tendency now for the nuclei to take a position just next to the plasma membrane. In figure 15 a later stage in which cleavage has gone still farther is shown. Cleavage is advancing also in the region of the columella but is markedly behind that on the periphery. Certain of the outer blocks have begun to subdivide by surface furrows at this stage. The planes of the cleavage furrows become very irregular as the process advances and the clefts which marked the course of the capillitial threads gradually disappear.

Tangential sections of these stages show the formation of the cleavage furrows very clearly (figs. 13 and 14). In the periphery of such sections the irregular blocks of protoplasm seem entirely free from each other, a little nearer the center the furrows are seen advancing and branching to cut off blocks of all sizes. Still deeper and at the center we find cross sections of rounded and angular cavities, which indicate the first stages in the formation of the cleavage furrows from the surfaces of the capillitial openings. The pushing out of the first clefts from these tubular capillitial cavities is very well shown in these figures. The cleavage is seen to be strictly progressive from the surface inward, and further the surfaces adjacent to the capillitial cavities are in their relation to cleavage exactly equivalent to the external surfaces of the mass of spore-plasm as a whole. The plasma membranes of the capillitial openings are the source of cleavage furrows to even a greater degree than the original surface plasma membrane of the spore sack as a whole. Figure 16 shows a drawing of a multinucleated block of the spore-plasm as it is being cut up into smaller fragments. It is plain here, as in the photographs, that the furrows cut into entirely undifferentiated protoplasm with no hyaline zones or rows of granules to indicate the direction they will take. Even in the last stages of cleavage in Didymium I have observed no such condensation of the spore plasm around the nuclei as one finds in Fuligo. The cleavage progresses in the same fashion until we have the condition shown in figure 17, in which both the outer surface of the spore mass and the inner surface have been cut up into small irregular protoplasmic blocks, while the middle region of the spore mass still shows large angular blocks of protoplasm which are being rapidly cut into by furrows over their entire surface. The ultimate result of this progressive furrowing is the formation of uninucleated rounded spores which are of fairly constant size, as shown in figure 18. They lie packed between the capillitial threads which still hold their radial direction running from the central columella to the peridium. The capillitial threads at this stage are so brittle that they break up to a considerable extent in sectioning.

As described, the segments cut off by the cleavage furrows in the earlier stages, here as in Synchytrium, Pilobolus, Fuligo, and many other forms, are multinucleated and of very varying form and size. As the cleavage progresses, however, it is apparent that the distribution of the nuclei, though irregular and superficially considered accidental, is none the less in so far definite that the cleavage furrows never cut off non-nucleated segments and in the end each spore contains a single nucleus. The final cleavage stages here, as I have described in detail for Fuligo, always cut two-nucleated blocks into one-nucleated spores. These are the definitive spores of the slime mould. They show no subsequent growth or nuclear division in the process of ripening.

The appearance of the cleavage figures suggests most strikingly the contraction which accompanies the process. In the period of most active segmentation the blocks of protoplasm lie quite separate from each other. Part of the open space as shown in the figures is doubtless due to shrinkage in fixation and in particular regions some segments have dropped out of the section. Still there can be no question that the free space between the blocks is to a considerable degree the result of the same extrusion of cell sap which led to the opening up of the broad surface furrows in earlier stages.

Rothert (27) long ago observed the escape of cell sap and accompanying shrinkage during spore formation in Saprolegnia. The conditions in Didymium with its radially placed capillitial threads on which in the early stages the extruded liquid accumulates in bead-like droplets resembling vacuoles are especially favorable for the demonstration of the parallelism between the exudation of water and the formation of the cleavage furrows. I have pointed out the evidence for a similar parallelism in the case of *Synchytrium decipiens*, where the extruded liquid contains substances which are blackened by osmic acid. Kusano (19) has observed the same extrusion of liquid in *S. puerariae* though denying that it is of universal occurrence in this species. The formation of vacuoles from which cleavage furrows may arise in Pilobolus and Phycomyces is undoubtedly a similar phenomenon bearing the same general relation to the cleavage process. Such loss of water from sporanges has long been associated with the ripening processes by which the watery protoplasm of the hyphae or plasmodium is transformed into the condensed and dust-like mass of spores. I have already pointed out the possibility that this exudation of water may at least be a factor in the process of cleavage on the assumption of an analogy between the furrowing of the spore-plasm and the cracking of a drying colloidal mass.

In a recent paper with Dodge (15) we have also pointed out the relation of this same extrusion of water into vacuoles in the early stages of the formation of the sporanges of Trichia to the formation of the capillitium. An essential and doubtless primitive feature of the transition from the expanded vegetative condition to that of the dust-like spores for reproduction in these slime moulds is the exudation of water and it is quite to be expected that from the physical chemical standpoint the formation of the capillitium, the production of thick spore walls and condensed reserve products, and even the process of cell division itself would all develop in a manner most intimately related with and dependent on the fundamental process of getting rid of superfluous moisture. Still as I have elsewhere emphasized cell division in these sporanges cannot be regarded as simply a matter of the drying out and cracking to pieces of a colloidal mass. The extrusion of water into internal vacuoles shows that the process is initiated and controlled by changes in the spore-plasm itself. The fact that the final furrows always separate uninucleated spores is also proof of organization in the segmenting mass.

Visible evidence of this organization such as we find in connection with the processes of cell division in many plant cells is entirely lacking in these sporanges. There seems to be no evidence whatever for the existence of such systems of specially oriented fibrils as are seen in the central spindle and polar asters and which play so important a rôle in the phenomena of cell division and free cell formation in the higher plants and in the ascus.

None of the mechanical theories of cell division like those of Heidenhain and Kostanecki, which also involve the existence of a special system of organic rays for each nuclear center, can have any application, as I have pointed out before, in cases of progressive cleavage of multinucleated masses such as we have in the spore sacks of the slime moulds and fungi.

Swingle (29) has clearly diagrammed the tension relations involved in the cleavage processes of these sporanges, whether or not the furrowing is really due to localized contraction of the cytoplasm, as he supposes. The cleavage furrows whether originating from the vacuoles, the periphery of the mass or the capillitial canals constitute a complex system of catenoidal surfaces which tend to divide the mass into spherical fragments. Such cleavage surfaces may equally well be conceived as due to exudation of water with increase of surface tension between the cytoplasm and the extruded moisture due to the ripening changes going on in the former, involving, as they probably do, condensation processes with change from highly hydrated proteids to lipoid or other fatty storage products. Such chemical changes may well play a part in the liberation of the energy necessary for the accomplishment of these profound form changes.

I have elsewhere suggested (14, 16) that in the gradual shrinkage and condensation of the spore-plasm the loss of water might be least in the neighborhood of the nuclei and that thus a determining factor in the orientation of the cleavage surfaces would be introduced. The well-known facts as to the effect of acids and alkalies on the imbibition of water by colloids utilized by Pauli (25) in his surface tension theory of the contraction of striated muscle fibers and by Fischer (12), in his theory of oedema may have significance in connection with those processes which involve the exudation of water in the sporange. A localized production of acid in the colloidal spore-plasm involving differentiated capacity for the retention of water might determine the direction of the cleavage furrows. The cleavage planes would naturally follow zones of greatest water loss thus isolating such acid-containing areas. If the nuclei either by reason of their characteristic chemical content (nucleo-proteids, nucleic acid) or by the products of their metabolism could thus become centers of moisture retention we should have a factor which would tend to such an orientation of the cleavage planes as in the end would produce uninucleated spore units. The chemical changes in the colloids in such acid regions might lead even to the visible differentiation of the hyaline zones which in the later stages of cleavage in Fuligo and the spore embryos of Pilobolus seem to predetermine the planes of the cleavage furrows.

Hofmeister has emphasized the possibility of widely different chemical processes and conditions coexisting in a polyphase colloidal system like the cell because of the low mutual diffusion rates of its various elements and the possibility of such differences in reaction between the nuclear region and the cytoplasmic region of the cell must be recognized. If we add to this conception of the nucleus as a center of water retention the further conception, suggested by its relation to cell plate formation in the higher plants and the ascus, that it is a center for the production of plasma membrane materials, we have two factors which would work in harmony to bring about the process of progressive cleavage as described, since the diffusion outward from the nucleus of substances to be used in forming the plasma membrane would again tend to cause the cleavage planes to pass midway between any given pair of nuclei.

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EXPLANATION OF THE PLATES XI AND XII

Figures 7 and 16 were drawn with the aid of the camera lucida from preparations stained with the triple stain. Zeiss apochromatic objective 3 mm. N.Ap. 1.3, ocular 8.

The microphotographs were made with the Zeiss apochromatic objective 16 mm. Oc. 4 with Cramer's micro-ray filter No. 7 except in case of the iron haematoxylin preparations, figures 15, 17, and 18.

PLATE XI

FIG. 1. Part of radial section of young sporange before cleavage, showing two capillitial threads, the vacuolated condition of the middle zone of the spore-plasm and the distribution of the nuclei.

FIG. 2. Entire horizontal radial section of sporange at stage when the spore-plasm has become quite homogeneous, showing radial capillitial threads and the distribution of the nuclei. Columella containing reticulated gelatinous material extruded in formation of the sporange. × 90.

FIG. 3. Horizontal section, later stage, showing rows of extruded droplets of cell sap on the capillitial threads. Columella showing lobed concretion. × 90.

FIG. 4. Like fig. 3. Well-developed concretion in columella. × 90.

FIG. 5. The droplets of cell sap have become ellipsoidal preparatory to flowing together. × 90.

FIG. 6. Horizontal section later stage with thin, watery sheaths around the capillitial threads. × 90.

FIG. 7. Drawing showing arrangement of the nuclei along the plasma membrane of the watery sheath surrounding a capillitial thread. × 800.

FIG. 8. Horizontal section showing a little later stage than fig. 6. × 90.

FIG. 9. Horizontal section showing the watery sheaths around the capillitial threads more strongly developed. Cleavage beginning at (*a*). The checking of the protoplasm is artefact.

PLATE XII

FIG. 10. Tangential section showing cleavage furrows forming about the capillitial openings. × 90.

FIG. 11. Stage a little later than shown in fig. 9. Cleavage at (*a*) and (*b*). × 80.

FIG. 12. Vertical section showing early stage in cleavage. × 90.

FIG. 13. Tangential section showing cleavage. × 90.

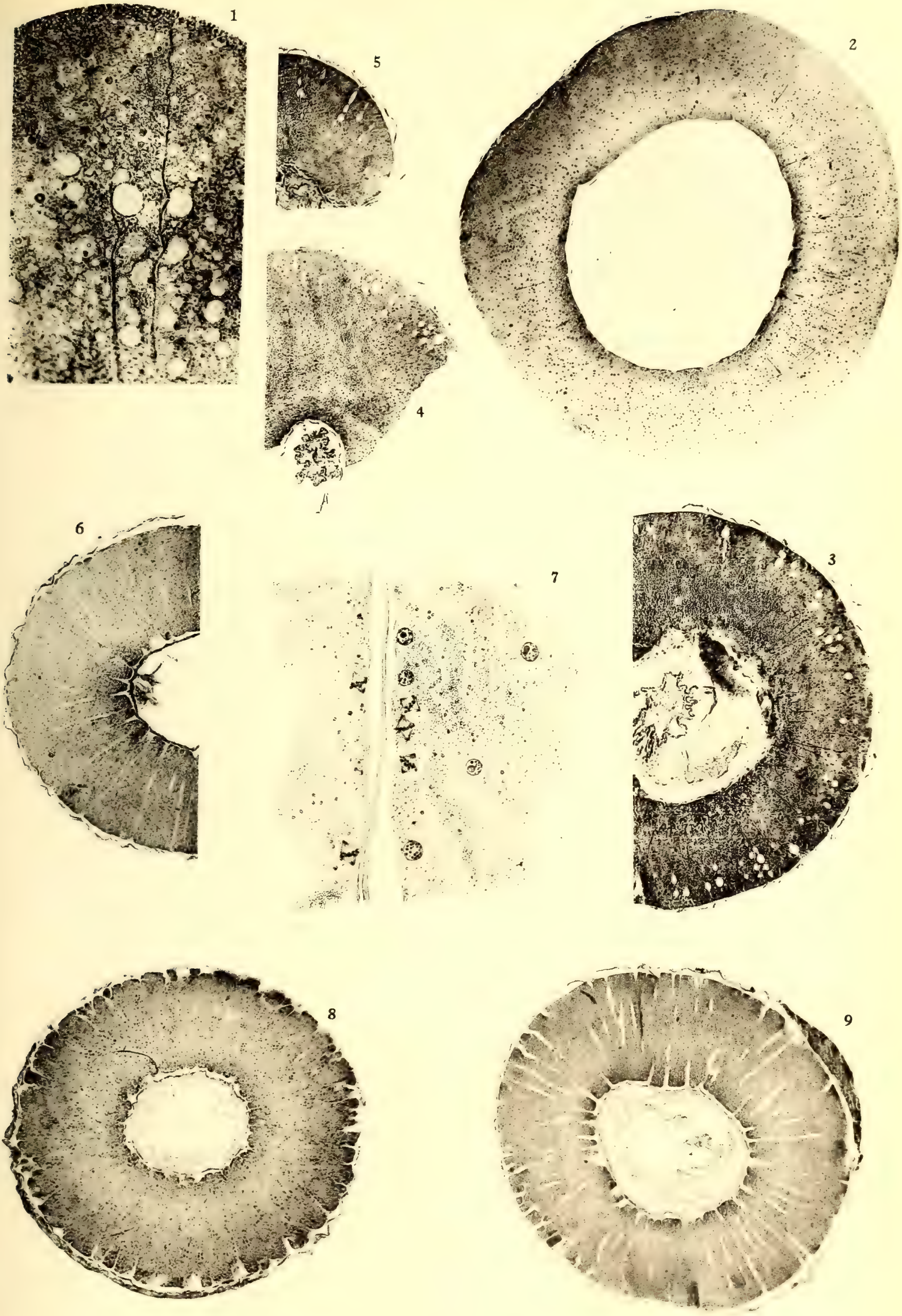
FIG. 14. Tangential section showing a little later stage than fig. 13. × 90.

FIG. 15. Advanced stage in cleavage. × 90.

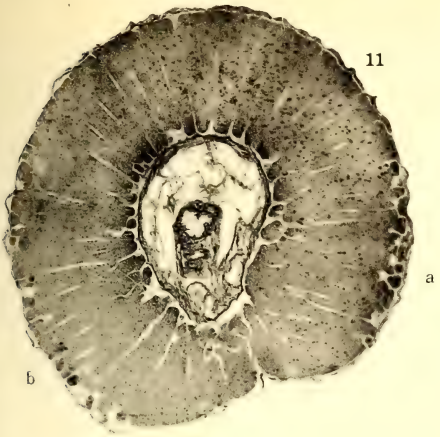
FIG. 16. Drawing of segmenting block of spore-plasm showing cleavage furrows and resting nuclei, others in equatorial plate stage. × 650.

FIG. 17. Late stage of cleavage. × 65.

FIG. 18. Vertical section of entire sporange showing spores, stipe and hypothallus. Stipe and hypothallus filled with granular concretionary material.



HARPER: CLEAVAGE IN DIDYMIUM.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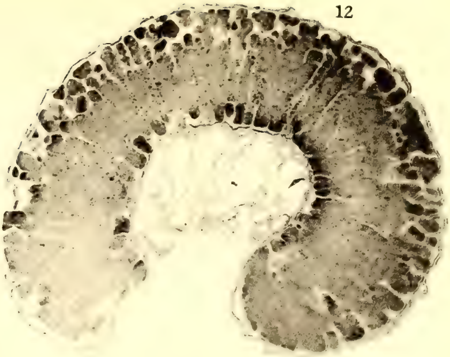
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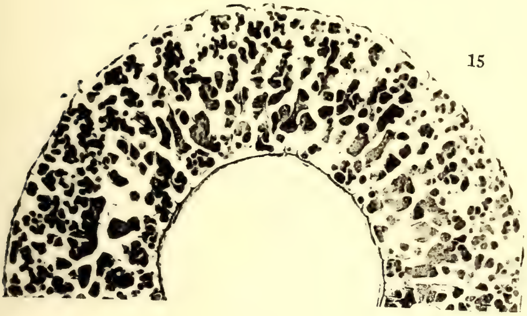
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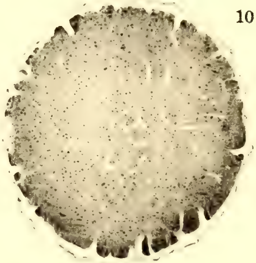
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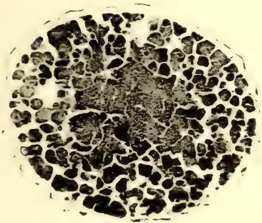
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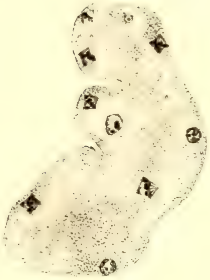
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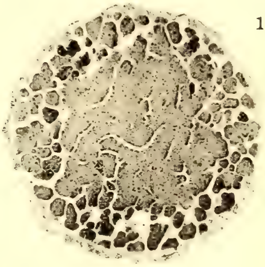
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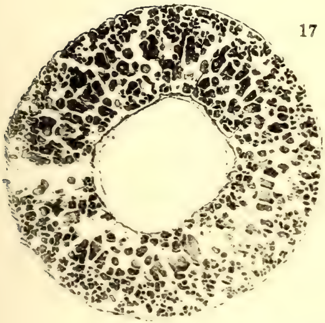
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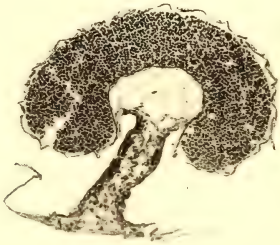
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HARPER: CLEAVAGE IN DIDYMIUM.

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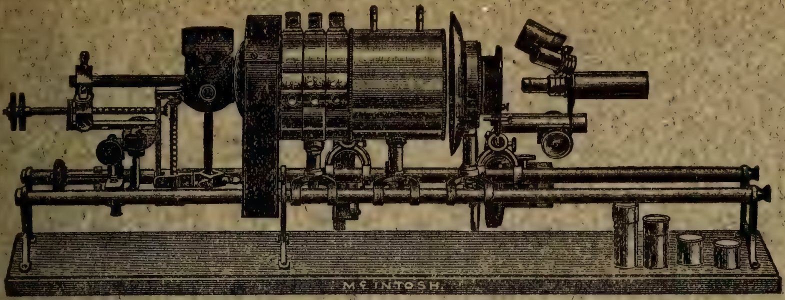

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CONTRIBUTION TO THE LIFE HISTORY AND PHYSIOLOGY OF CYLINDROSPORIUM ON STONE FRUITS[1]

BASCOMBE BRITT HIGGINS

WITH PLATES XIII–XVI

INTRODUCTION.

The disease of plums and cherries caused by the fungus Cylindrosporium has long been known, and because of its economic importance and peculiar appearance has for many years attracted the attention of horticulturists and plant pathologists both in Europe and America. On certain species of the hosts the disease becomes very conspicuous because of the "shot hole" effect on the leaves, produced by the dropping out of roundish areas of diseased tissue. From the leaves of other species however the spots do not drop out, and usually in such cases the leaf tissue is not killed to any appreciable extent. In cases of severe attack the leaves often turn yellow and drop prematurely, which of course interferes more or less seriously with food production and the future welfare of the tree.

"Shot holes" are produced in the leaves of stone fruits by several different organisms or even by mechanical injury, for example, as the result of a needle prick. Duggar (8) found also that they were produced readily by spraying plants with poisonous solutions. However, the fungus Cylindrosporium is probably the most prolific cause of this phenomenon, at least in cherries; and many collectors have apparently attributed all "shot hole" effects on species of Prunus to *Cylindrosporium padi* Karst.

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[1] Contribution from the Department of Botany, Cornell University. No. 155.

[The JOURNAL for March (**1**: 97–144) was issued 6 May 1914.]

This fungus was described by Karsten (17) in 1884 from leaves of *Prunus padus;* and Sorauer (13) states that in Europe the disease is confined almost entirely to this species. Aderholdt (1) in 1901 said that the disease had been common on both sweet and sour cherries during the previous ten years. In America a disease attributed to this organism has been reported on nearly all species of Prunus both wild and cultivated.

Because of the prevalence of the disease and its consequent economic importance, it has seemed very desirable to know the complete life history of the fungus causing it. With this purpose in view an investigation has been in progress during the past three years, the results of which are here reported.

For several years the disease has been very abundant in the vicinity of Ithaca, New York, on the sweet cherry (*P. avium*) and the wild choke cherry (*P. virginiana*). It has been found less abundant on the sour cherry (*P. cerasus*, *P. mahaleb*, *P. pennsylvanica*), and on the plums (*P. domestica*, *P. insititia*, and *P. spinosa*). Through the courtesy of Professor J. G. Hall (now of Pullman, Washington) leaves of *P. serotina* affected with the disease were obtained from Clemson College, South Carolina, in August, 1912. A Septoria having spores only slightly different, but produced in a pycnidium, also found on *P. pennsylvanica*, was studied for comparison with these. The results of this study of Septoria will be reported in another paper.

This abundance of material on a number of host species has made possible a comparative study of structural characters, relation to the host tissue, cultural characters, interrelations of the fungus on the different hosts, and its life history. Pure cultures of the organism from *P. spinosa* were not obtained, so that it could not be included in all the comparisons.

Structural Characters

The genus Cylindrosporium is characterized by having elongated colorless conidia borne on a more or less disk-shaped stroma just beneath the host epidermis. The acervuli on all the species of Prunus under observation agree with this characterization. The stroma is very delicate, at first consisting of one cell layer only, but becomes slightly thicker as the acervulus grows older. On the outer surface of this stroma short conidiophores give rise to conidia. They develop first over the center of the stroma and continue to develop centrifugally as the latter grows in diameter. This growth of the stroma is lateral, extending out between the epidermis and mesophyll of the leaf, and never turns up at the edges so as to resemble a pycnidial structure.[2] The stroma which bears conidia lies under the epidermis on either surface of the leaf. When the conidia have accumulated in sufficient number the epidermis is broken and they appear usually as a whitish or yellowish white mass above the stroma. In *P. serotina* (more rarely in other species) the stiff cuticle prevents the formation of a large opening and the conidia are forced out in long tendrils.[3]

The conidia from all the host species are very similar. They are long, slender, curved or flexuous, and continuous or 1–3 septate.[4] There are a few minor differences which are not very distinct, since the conidia vary considerably on each host. The conidia from plum leaves (all three species) were more constant than those from cherry leaves. Here they are blunt at the proximal end and taper gradually toward the apex, and they are mostly once septate. The largest spores found were from *P. serotina* and *P. virginiana*. On both species spores measuring 45–80μ in length were found. On no other species were they found as long as 80μ.

---

[2] *Septoria cerasina* Peck (29th Report N. Y. State Mus. Nat. Hist. 48, 1878) the type material of which, through the kindness of Dr. Peck, I have had an opportunity of examining, has a typical fruiting structure of this description (fig. 4). The same is true of the specimen distributed in "Fungi Americana" No. 747 as *Septoria Ravenellii* Thüm, which is apparently identical with *S. cerasina* Peck. This is not the case, however, with *Septoria pruni* Ellis, which Aderholdt (1) includes as a synomym of *Cylindrosporium padi* Karst. Here a distinct pycnidial structure surrounds the spores which shows it to be a true Septoria. This was not from type material, but from the specimen in Ellis' North America Fungi 1151 in the Cornell University herbarium. It is from the same collection which Aderholdt must have examined, though of course in such a large collection some leaves affected with Cylindrosporium might also be included.

[3] This accounts for Peck's (23) statement that *S. cerasina* differs from *Cylindrosporium padi* (on *P. domestica*) in this character.

[4] Aderholdt (1) says that the conidia are not truly septate but have false cross walls. He does not state how this was demonstrated. Pammel (22) says that the cross walls react to stains in exactly the same way that the side walls react, and this has been confirmed by my own studies. Haidenhain's iron alum haematoxylin Delafield's haematoxylin, and Flemming's triple stain all bring out the cross walls very distinctly. Very often also the cells become constricted at the cross walls upon germination; or the contents of one cell may be lost entirely without apparently affecting the rest of the conidium.

Cultural Characters

The conidia seem to lose their vitality very rapidly on drying. When they are taken from the dry masses which cover the acervuli only a small per cent of them germinate in nutrient agar or in tap water; and some taken from cherry leaves which had not been wet for about a month failed to produce infection on other leaves of the same plants.

Fresh spores germinate very slowly in agar. For this reason and because of the large number which failed to germinate it was found very difficult to obtain pure cultures of the fungus by the dilution method. When leaves were placed in a moist chamber for a few hours so that fresh conidia were formed, pure cultures were readily obtained by picking up a quantity of them on the point of a needle and then dragging the needle across an agar plate. After four or five days there were usually spots on some of the streaks free from bacteria and other fungi where some of the Cylindrosporium conidia were beginning to germinate. Blocks of agar containing such germinating conidia were then transferred to tubes of agar or to sterilized bean pods.

Comparison of the fungus isolated from the different hosts was made chiefly from growth on steamed bean pods; but the nature of the medium seemed to have very little effect on the nature of the growth, colonies very similar to those on bean pods being produced on agar dissolved in tap water; on agar to which had been added an extract of beans, potatoes, malt extract, prunes, or cherry leaves; on sterile slices of pear; or on steamed cherry leaves.

Growth from the spores is always very slow, and is usually not apparent to the unaided eye until 10–15 days have passed. At this time it appears as a small whitish speck, which when examined microscopically is found to consist chiefly of a stroma covered with quantities of conidia similar to those produced on the host plant. This stroma grows slowly, enlarging until a hemispherical mass 0.5–1 cm. in diameter is formed, which occurs in about two months. Before this time the stroma has turned coal black and has a carbonaceous, crust-like appearance in the fungus isolated from *P. domestica* and *P. insititia*. After the stroma has turned black few spores are produced even when transferred to new media. The stroma produced from the fungus isolated from *P. avium*, *P. cerasus*, and *P. pennsylvanica* sometimes turns dark but never black and crust-like as in that from the plums. The stroma from the other three cherries, *P. virginiana*, *P.*

serotina, and *P. mahaleb*, has never been observed to turn black and has usually a creamy white and more floccose appearance.

Relation between Fungus and Host

Interest in the physiological relation between the fungus and the host tissue was aroused by the observation, before mentioned, that the spots containing Cylindrosporium are deciduous in some species while in others they are persistent. Even in the same species there is marked variation in this respect. This variation is very striking in *P. virginiana* where ragged remnants of leaves, from which dozens of spots have dropped out, may later be abundantly infected with apparently little injury to the leaf tissue except the killing of a few cells in immediate contact with the acervulus. Because of this marked variation as host, *P. virginiana* was used chiefly in this part of the investigation which was undertaken in the hope of finding some explanation for this phenomenon.

The first problem was to find, by a histological study, what occurs just before and at the time of the dropping of the spots. For this purpose a series of spots were cut out so as to include some of the surrounding healthy tissue. These were killed, embedded in paraffin, and sectioned. The series began with the first sign of infection (a slight yellowing of the leaves) and included all visible changes until after the spots had fallen. After sectioning, several stains were tried. Durand's (9) method for differentiating mycelium of parasitic fungi worked very well, and was used where it was desired to see the position and extent of the mycelium.

The mycelium is intercellular, with haustoria which penetrate the host cells (figs. 17, 18). The haustorium enters through a very small hole in the cell wall and is very much attenuated as it enters, but the end enlarges into an oval or elliptical body which contains a nucleus and a comparatively large vacuole. After the haustorium has entered, the protoplasm of the invaded cell often deposits a cellulose sheath around the haustorium, apparently similar to that formed around the haustoria of the Erysiphaceae as described by Smith (25). This sheath often extends along the wall of the host cell for some distance also.

The host cells are not killed at first except those in contact with the stroma; and their death is probably brought about by drying rather than as a result of any toxic secretion from the fungus protoplasm. That no very poisonous toxin or enzyme is given off is indicated by the fact that cells penetrated by the haustoria appear healthy and are able to deposit a cellulose sheath around the haustorium.

In every case examined acervuli had already formed and had broken the epidermis of the leaf, more commonly on the upper surface, but often on both surfaces. In every case also, even the very earliest, the tissue of the spot containing the fungus had already separated or begun to separate from the surrounding tissue.

Formation of Absciss Layer Around the Spots.—This separation is brought about by the enlargement of a layer of cells at some distance from the ends of the mycelium (figs. 14, 15, 16). Their enlargement is so abrupt and so great that the active cells separate from the adjoining inactive cells inside. The enlarged cells have lost their chloroplastids and nuclei, and only a thin layer of protoplasm lines the wall. The loss of the chloroplastids causes the watery appearance of this ring of tissue which was noted by Duggar (8). The tendency for the formation of this layer to follow the veinlets of the leaf as mentioned by this author has very rarely been noticed. The spots which drop out are usually very nearly round; and, even when quite irregular, the margin is evenly curved and smooth, not angular as it would be if the separation was limited by the veinlets.

After the separation is completed all around, the spot turns yellow, shrivels rapidly and soon drops out. Sometimes (often in *P. serotina*) the tension exerted by the enlarging cells is not sufficient to rupture the cuticle, but the cellular tissues are ruptured and the spot dries but remains in position. After the separation has occurred a layer of cells just outside the enlarged cells shrivel, forming additional protection for the healthy tissue.

In order to obtain the earlier stages of the process, plants in the greenhouse were inoculated with fresh conidia from leaves of *P. virginiana* which had been inoculated with a pure culture from the same species. The conidia were placed in a drop of water on a glass slide and a little powdered chalk added for the purpose of marking the inoculated spots. Small droplets of this water containing spores and chalk were placed on the under surface of the leaves of two plants and on the upper surface of two, and all four plants covered with bell jars. After three days some of the inoculated spots were cut out, killed in hot (about 60° C.) chrom-acetic acid, and embedded in paraffin. This was repeated every 24 hours until acervuli appeared above the chalky spots on the sixth day. Infection did not occur in any case where the spores were placed on the upper surface of the leaves.

When this paraffined material was cut and stained no mycelium was found within the leaf tissue killed the third day. By the end of the fourth day some germ tubes had entered and had formed quite extensive mycelium. When these mycelial threads, entering the stomates of the lower epidermis and traversing the mesophyll, come in contact with the upper epidermis they branch profusely and form by the end of the fifth day a very delicate stroma with conidiophores and young conidia. By the end of the sixth day conidia have matured in sufficient numbers so that the pressure ruptures the leaf epidermis above the stroma.

No haustoria were found until the fifth day. The host cells appear normal until the sixth day, when, in a few cells just beneath the stroma and in contact with it, the protoplasm was slightly shrunken, due probably to drying. This appearance spreads rather rapidly after the epidermis is broken. The leaf tissue containing the fungus is separated off by the enlargement of certain cells mentioned above and the separated tissue yellows rapidly. The length of time varies, but usually within 7–10 days after infection the spots begin to yellow and drop out.

An apparently related case to this shedding of the diseased spots is that reported by Galloway (12) in which the needles of *Pinus virginiana* drop off after the formation of Coleosporium pustules. Galloway says that this casting is not caused by the fungus directly but by the loss of water through the break in the epidermis.

Enlargement of Cells of Absciss Layer.—To explain the enlargement of the cells of the separation layer, four hypotheses have been suggested, viz.: *First*, the release of tension due to shrinking of the adjoining cells may allow the cells to enlarge; *second*, the cell walls may be softened by some enzyme secreted either by the fungus or by the host protoplasm, thus allowing the cells to expand; *third*, the colloids of the cell (protoplasm, cell wall, etc.) may be so modified as to have a greater affinity for water; and *fourth*, the osmotic pressure may in some way be increased in these cells. Of the four the last seems to be the most plausible, and while it is not yet proven beyond doubt all observations seem to support this as an explanation for the phenomenon.

The first hypothesis is invalid because these cells begin to enlarge before the adjoining cells have shrunken to any appreciable extent. Further the cells of these leaves do not enlarge to any appreciable extent, if the tension is removed entirely by cutting or tearing the leaf or if the tissue is macerated and the cells set free in water.

The second hypothesis does not appear valid, since if such an enzyme were secreted it would probably spread to adjacent cells. Also it is not very likely that, with the loss of water from the adjacent cells and consequently to some extent from these, the internal pressure would be great enough to expand the cells very much. At least with only ordinary osmotic pressure to hold the cell water, the cells would quickly collapse on exposure to the air.

The third hypothesis is similar to that held by Fischer (10) to be the cause of oedema in animals, where the swelling is said to be due to modifications in the fibrin of the blood, muscle, etc. At first the disappearance of the plastids and nuclei seemed to argue in favor of this hypothesis, but when the stained sections were examined again the interior of the cells was found to be colorless while the protoplasm lining the cell wall was stained and distinct, which indicates that the interior is filled with cell sap.

That osmotic pressure is capable of causing the enlargement of plant cells is shown by the development of intumescences under various conditions during which increased osmotic pressure in the cells occurs. The question is then, how can the increased pressure be brought about in this case.

Production of Shot Holes Correlated with Amygdalin Content of Leaves.—Observations seem to indicate that the shedding of the diseased spots is correlated with the amygdalin content of the leaves. As before mentioned the spots are shed from *P. virginiana* during all the spring and early summer. Morse and Howard (20) have shown that the young leaves of this species are very rich in amygdalin which diminishes in amount with the age and vigor of the leaves. Of the species under observation, the following also shed the spots infected by Cylindrosporium while the leaves are young: *P. serotina*, *P. pennsylvanica*, *P. cerasus* and less frequently *P. domestica*. They are never shed from leaves of *P. mahaleb*, *P. spinosa* (not seen infected while leaves were very young) and, except under rare conditions, from *P. avium*. According to publications summarized by Wehmer (30), amygdalin is found in the leaves of *P. virginiana*, *P. pennsylvanica*, *P. serotina*, *P. cerasus* (?) and in young but not in mature leaves of *P. domestica*. It is not found in *P. mahaleb*, *P. spinosa*, or *P. avium*.

It has been found that leaves which contain amygdalin produce also an enzyme, emulsin, which, under certain conditions, breaks the amygdalin down into benzoic aldehyde, hydrocyanic acid and glucose.

Morse and Howard (20) found that wilted cherry leaves yielded much more prussic acid than fresh leaves, but offer no explanation as to why the leaves should do so.

More recently H. E. and E. F. Armstrong (2), in an interesting paper on "The origin of osmotic effects," find that prussic acid is set free in the leaves of the cherry laurel by treating the leaves with vapors of anaesthetics, with alcohols, or many other organic and inorganic compounds which enter the cells of the leaves. The authors think that when these substances enter the cells they change the osmotic relations in the cell, and enzymes are set free which break down the hydrolites stored there.

Guignard (14), who has done much work on emulsin and cyanogenesis, found emulsin in the endodermis of the vascular bundles only in leaves of *P. laurocerasus*. He suggests that wilting, action of chemicals, anaesthesis, or anything which alters the osmotic relations of the cells would bring the emulsin and hydrolite together.

Mann (19)[5] states that, in curing certain grades of tea, leaves allowed to wilt slowly increase the amount of the enzymes which break down the objectionable compounds. He thinks that these enzymes are not present as such, but zymogens are present which break down and form enzymes when the leaves wilt.

Green (13) thinks that in many of the plants enzyme antecedents exist first as zymogens, and in a few instances has shown rather definitely that such is the case.

It is quite possible that the emulsin zymogen exists, along with amygdalin in the cells of cherry leaves, and that the enzyme is set free by slight changes in the osmotic pressure in the cell. Should this be the case certain observed phenomena (e. g., the rapid splitting of amygdalin in wilted leaves) could be more readily understood.

In whatever condition the enzyme exists, in cherry leaves it seems certain that it comes in contact with and breaks down the amygdalin when the leaf tissue wilts; and with this fact in mind a very plausible theory of "shot hole" formation can be formulated. When the acervuli and spores of Cylindrosporium break the leaf epidermis, the underlying cells dry out rapidly. As the wilting spreads the amygdalin comes in some way in contact with the emulsin and is broken down. Each molecule of amygdalin breaks down into four molecules (one each of hydrocyanic acid and benzaldehyde and two of glucose), thus materially increasing the osmotic pressure in the cells where this occurs. The increased osmotic pressure enables them to draw water from the adjoining cells and swell until this pressure is more nearly equalized.

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[5] Original paper not seen but only a summary in Fowler's "Bacteriological and Enzyme Chemistry."

Since HCN has been found to increase the activity of proteolytic enzymes, it is quite probable that its presence in the enlarging cells causes the digestion of the plastids. Butkewitsch (7) found that addition of HCN accelerated the self digestion of the proteins in crushed seeds of several plants. Vines (29) also noted this property of HCN and suggested that its function in seeds might be to facilitate proteolysis of the reserve materials on germination.

Amygdalin Removed from Area of Leaf Occupied by the Mycelium.—One question which naturally arises is, Why is this separation layer always formed at such a distance from the acervulus as to include the fungus mycelium? This question is readily answered according to the previously discussed theory, if the fungus is capable of using amygdalin as a food; since in that case the amygdalin from the cells in the immediate neighborhood of the mycelium would be absorbed by it. The separation layer would then be formed by the cells outside this region, the amygdalin of these cells breaking down and producing the necessary osmotic pressure. The fungus could certainly use a part of the amygdalin molecule (e. g., the sugar), if it contains an enzyme to split the amygdalin into these simpler compounds.

To test this hypothesis, several cultures of the fungus from *P. virginiana*, *P. mahaleb*, *P. pennsylvanica*, and *P. avium* all growing on sterilized bean pods were used. From four cultures the liquid in the bottom of the tube was filtered off and a quantity of absolute alcohol added to the filtrate. A flocculent precipitate was soon formed. The liquid was again filtered, the precipitate washed with a mixture of ether and alcohol, dried and redissolved in 8 c.c. of water. The liquid from each tube was thus treated separately. As checks, the extract from two of the cultures was boiled and to each of the four, 2 cc. of a 5 per cent solution of amygdalin was added. A few drops of chloroform were added to each as an antiseptic. The preparations were then corked and set in a locker in the laboratory. After

18 hours there was an almond odor in the tubes containing the unboiled extract and by the end of 8 hours this odor was very strong. At this time they were tested for reducing sugar and for HCN. Both were present in the tubes containing the unboiled extract while the checks gave no trace of HCN and only a slight trace of reducing sugar. The other cultures were crushed in a mortar, extracted with 30 per cent alcohol, filtered, and the filtrate treated in the same way that the liquid from the other tubes had been treated. In every case emulsin was found to be present in the unboiled extract.

For comparison with the behavior of Cylindrosporium and its hosts, a Septoria found on leaves of *P. pennsylvanica* was studied. In this case the mycelium passes directly through the host cells and kills the host tissue before fruit bodies are formed. The spots drop out less frequently than Cylindrosporium spots on the same species. Where they do drop out however there is a separation layer formed in the living tissue just outside the dead tissue. A similar separation layer was also found in leaves of *P. americana* affected with Cercospora. (From herbarium material distributed as *Cylindrosporium padi* Karst. in Griffith's "West American Fungi" 75*a*.)

Frank (11) also found that such a callus layer is formed around spots infected by *Gnomonia erythrostoma* Pers., and Duggar (8) noticed its formation in leaves of Prunus in which "shot hole" was produced by poisonous compounds.

DEVELOPMENT OF THE PERFECT STAGE[6]

The perfect or ascigerous stage of the fungus on *P. avium* was found in the spring of 1911 and its development was studied more closely during the winter and spring of 1912. From the knowledge thus gained it has been comparatively easy to find and follow the development of the perfect stage on the other hosts under observation, especially since trees whose leaves were infected with Cylindrosporium were located during last summer (1912). It has been found on all the hosts under observation except *P. spinosa*, of which only a few leaves were found infected with the conidial stage last summer.

There is considerable variation in the perfect stages found on the different host species, but they may be divided into two main groups according to the shape of the ascocarp and its position in the leaf. Since the development varies slightly in the two types, the development on *P. avium* will be described and the others compared with this one.

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[6] A brief description of the structure and development of the perfect stage of Cylindrosporium on *P. avium* was given in a previous note (16); but since many points of interest could not be included in a brief note, the entire development will be given in more detail here.

On this host the production of normal Cylindrosporium conidia (macroconidia) ceases usually early in August, and from this time until after the leaves fall minute conidia (microconidia) about one tenth the length of the macroconidia are formed in great numbers. They are abstricted from the apex of short branched conidiophores (fig. 39) which appear to be the same ones on which the macroconidia were produced earlier. At least they are on the same stroma and in similar positions.

Almost simultaneous with this change in spore form, the stroma begins to develop downward through the mesophyll and palisade layers of the leaf. This growth is at first composed of separate but profusely branched and rather tightly packed threads; but an outer pseudoparenchymatous layer is later differentiated. The host cells are often surrounded by this mycelial growth and often, especially the lignified cells of the vascular bundles, remain so enclosed until the fruit body matures the next spring (figs. 6, 8). Before the formation of the pseudoparenchymatous covering, coils of densely staining hyphae appear in the stroma. Several of these coils, often six or eight, are formed in each stroma. Each coil consists of two or three turns in the hypha and is made up of several uninucleate cells. Its free end is extended as a trichogyne-like structure, which is also several-celled and slightly enlarged at the apex. This swollen tip extends above the surface of the stroma and ends just above the layer of conidiophores from the apex of which the small conidia (spermatia ?) are being abstricted. Soon after leaf-fall the trichogyne-like structures disintegrate and very soon disappear entirely. The fate of the coiled base of this structure is as yet an open question. The dense pseudoparenchymatous covering, which about this time develops entirely around the stroma, makes the inner portion extremely difficult to fix satisfactorily. In material killed November 7 in Flemming's weak osmic acid fixer, the coils show decided signs of degeneration. They are stained scarcely at all by Heidenhain's iron alum haematoxylin, when the surrounding cells are deep black. Also in some of the material killed later no sign of the coils can be seen. On the other hand, in some material from the same tree killed December 28 in Carnoy's alcohol acetic acid fixer, many stromata show apparently healthy coils. Later than this they were not seen with certainty.

The pseudoparenchymatous covering of the stroma separates the layer of microconidiophores which now gelatinize and glue the remnants of the epidermal cells to the surface of the stroma. The cells of the pseudoparenchymatous covering as well as the mycelium in the leaf tissue become thick walled and dark colored.

The stroma usually extends entirely through to the upper epidermis, but remains covered both above and below by the leaf epidermis. It is apparent only because of the dark color, and in this condition the fungus passes the winter.

During the first warm days of March the stroma begins to swell toward the lower (dorsal) surface of the leaf, and when sectioned this swelling is seen to be due to a row of erect parallel hyphae which later are seen to be paraphyses. The asci do not appear until about the first of April or later. They develop from branched ascogenous hyphae which arise near the base of the stroma. During the latter part of April and the first of May the asci enlarge rapidly and lift the covering until it finally breaks in a more or less stellate manner. The break occurs before the ascospores are mature, but they mature in a very short time thereafter.

The asci open by a pore in the papillate apex and the spores are shot out. On taking leaves, in which the ascospores are just mature, from a moist chamber, clouds of spores have a few times been seen shot out from the under surface.

After the ascospores are shed the asci and paraphyses disappear, and long slender conidia are formed on short conidiophores which arise apparently as branches from the base of the paraphyses. They are once or twice septate and resemble Cylindrosporium conidia but are usually longer and a little more slender.

Besides being on *P. avium* this type of fruit body and development was found on *P. pennsylvanica* and *P. cerasus.*

In the other type, found on *P. domestica, P. insititia, P. virginiana, P. serotina* and *P. mahaleb*, the stroma develops beneath the lower epidermis and does not extend into the leaf tissue to any appreciable extent. The development is more outward, thus protruding and forming rather prominent disk-shaped to flattened-globose bodies on the under side of the leaf.

In the leaves of *P. serotina*, *P. virginiana* and *P. mahaleb* the stroma never becomes black but changes to a yellowish brown or to a dull orange color with a waxy appearance when wet. The paraphyses are differentiated much earlier (in January or February) and the ascospores mature slightly earlier. Leaves of *P. serotina* received from Clemson College, S. C., on March 13, bore mature fruit bodies. In leaves from the same tree received in the fall and wintered over in wire cages (Ithaca), they did not mature until about a month later.

On the plum (*P. insititia* and *P. domestica*) the stromata often turn coal black before the leaves fall. They protrude from the under surface of the leaf and because of the black color are very prominent all during the winter. The spring development is much slower and later than in *P. virginiana*, and slightly later than in *P. avium*. In all other respects the general development as far as observed was similar to that described on *P. avium*.

Infected leaves of all the host species were brought into the laboratory at intervals during the fall and winter. For some reason, however, the fruit bodies refused to develop if brought in before the paraphyses had been differentiated. Perhaps the excessive moisture, or else lack of freezing, prevented the formation of the ascogenous hyphae. It was also noticed that the fruit bodies failed to develop outside when the leaves were packed closely together and therefore moist and poorly aerated, and also when a leaf was folded so that half of its under surface was next the ground the fruit bodies failed to develop on the side in contact with the ground.

When plum leaves were kept very moist in a closed moist chamber the asci did not develop, but conidia were formed instead. This may have been due to the inherent nature of the fruit body, but that it was due to the effect of excessive moisture is more probable. Leaves which had been kept very wet until most of the fruit bodies had developed into conidia-bearing structures, were gradually dried by exposing to the air for a few minutes each day when normal asci and paraphyses developed.

Relation of the Ascigerous Stage to Cylindrosporium

In order to remove all doubt as to the genetic connection between this ascogenous fungus and the Cylindrosporium which is parasitic on the several host species, several series of inoculations and cross inoculations were made during the early spring of 1912 and 1913. The inoculations were all made in the greenhouse and mostly before the ascospores had developed outside.

Species of Prunus Employed in the Inoculations.—The plants used for inoculating in 1912 were imported seedlings of mazzard (*P. avium* furnished by the J. B. Stewart Nursery Co.) and small trees of *P. serotina* obtained from a nearby thicket. These were cut back to mere stubs, dipped for a few minutes into a 7 per cent $CuSO_4$ solution, planted in small pots, and set in the greenhouse.

In 1913 these plants were given the same treatment and used again. About 50 trees each of *P. virginiana* and *P. pennsylvanica* and 15 of *P. americana* obtained growing wild in this region; 50 mahaleb cherry and 50 myrobalan plum trees furnished by the Greening Nursery Co.; 50 sour cherry (Early Richmond) and 50 peach (Elberta) trees bought from a local nursery; and 100 plum trees, 25 each of *P. domestica* (several varieties), *P. americana* (several varieties), *P. hortulana* (several varieties), and *P. insititia*, furnished by the Horticultural Department of the Geneva Experiment Station were given the same treatment.

The trees were cut back so as to give vigorous healthy leaves for inoculating and also so they could be covered with bell-jars when this was desirable. They were dipped in the $CuSO_4$ solution to kill any conidia of Cylindrosporium as well as spores of other fungi which might interfere with the results of inoculations, although in so far as Cylindrosporium is concerned sterilization was probably unnecessary, since all observations indicate that the conidia are very short lived and are unlikely to live over winter. None of the plants were ever attacked by Cylindrosporium unless inoculated, although often kept as checks under conditions very favorable for infection had conidia been present.

The plants when used for inoculation had vigorous shoots with usually 12–50 leaves. The myrobalan plums however were so slow in starting that only a few small plants were ready when the last inoculations were made.

Inoculations with Conidia.—The first series of inoculations was made for the purpose of determining the conditions most favorable for infection. Four plants of mazzard cherry were inoculated with a pure culture of Cylindrosporium from the same host species. The conidia were shaken up in a small amount of sterile water which was then placed in small droplets on the upper surface of the leaves of one plant, and on the under surface of the leaves of the other three. The former and one of the latter were then covered with bell-jars. Two of those with conidia on the under surface of their leaves were left in the open air of the greenhouse, but moist absorbent cotton was wrapped around the leaves of one plant.

At the end of ten days the plants were examined, when several Cylindrosporium acervuli were found on the two plants under bell-jars, a few on the plant whose leaves were wrapped with moist cotton, but none on the plant whose leaves were left exposed to the air.

This showed clearly that the leaves must be kept moist for satisfactory infection to occur; so the bell-jar method was used in all later inoculations except where otherwise stated.

In the next series ascospores were used. Ascocarps which had matured on leaves placed in a moist chamber March 16 were on April 4 crushed in a small amount of sterile water which, after being examined microscopically and found to contain quantities of ascospores, was used for inoculating several plants. When examined 7 days later numerous small white specks, which proved to be acervuli of Cylindrosporium, were found on the under side of the leaves.

On April 16 two more plants were inoculated in the same way and at the end of eleven days 45 acervuli were found on their leaves.

To determine whether conidia produced in the ascocarps also served to propagate the fungus, these were used to inoculate some plants. The conidia, if leaves are kept moist after the ascospores are shed, collect in small white clusters on top of the old ascocarps. Several of these clusters were picked up with a needle and distributed in drops of water on sterile glass slides. The water was then examined under the microscope and found to be apparently free from ascospores which can be distinguished by their smaller size and blunt ends. The two plants which were then inoculated with these conidia showed several acervuli at the end of seven days.

Because of the difficulty of obtaining any mycelial growth, no pure cultures were ever obtained from these apothecial conidia from *P. avium.* They frequently germinated in water and in agar but when the germ tubes had reached less than half the length of the spore, growth ceased.

Inoculations with Ascospores.—The ascospores behaved in much the same way. Several hundred cultures were made during the spring of 1911 and 1912 before any growth was obtained. Spores were often found germinating but when transferred to fresh agar, sterilized bean pods, or sterile slices of pear, no further growth occurred. Bean, potato, malt extract, prune, cherry leaf, and plain agar (agar dissolved in tap water), were tried. The plain agar was finally adopted for all germination trials since other fungi and bacteria developed to a much less extent and the ascospores (which germinated more freely than in the other agars) could more often be obtained free from contamination. Finally from a single lot of cultures 37 colonies developed from germinating ascospores transferred, some to tubes of plain agar, others to steamed bean pods and slices of pear.

The growth was very slow. Nearly a month elapsed before any growth could be seen with the unaided eye. The colonies developed as small oval masses similar to those of Cylindrosporium. At first great quantities of conidia were produced, but after about two months conidial production ceased and the stroma turned dark in most of the colonies. Transfers from this stromatic material gave few conidia so that difficulty was experienced in obtaining infections the next winter.

The first inoculations with these pure cultures were made Feb. 1, 1913. A colony from a steamed bean pod was crushed in some sterile water and used to inoculate two plants. After two weeks no infection was found and the same plants were again inoculated this time with spores produced on a very young colony. The drops of liquid left discolored spots where placed on the leaves and after seven days 40 of these spots showed Cylindrosporium acervuli.

Ascocarps were not found on the other species of Prunus until the spring of 1913; so, although several attempts have been made, no pure cultures have been obtained from them. Successful inoculations have been made with ascospores from leaves of *P. virginiana*, *P. serotina*, *P. pennsylvanica*, *P. domestica*, and *P. insititia* and with a pure culture from apothecial conidia from *P. insititia* infection has been produced on *P. americana*.

In fact the ascospores are more active in producing infection than the conidia of Cylindrosporium. Ascospores from leaves of *P. virginiana* produced abundant infection on plants of *P. serotina* and *P. virginiana* while conidia formed on the *P. virginiana* plants from this inoculation would not cause the disease on *P. serotina* although several plants were inoculated under exactly the same conditions, and abundant infection occurred on a plant of *P. virginiana* inoculated at the same time. In like manner the ascospores, but not the Cylindrosporium conidia from *P. serotina*, would infect *P. virginianum* or *P. mahaleb* plants.

Plants of *P. serotina*, *P. virginiana*, and *P. americana* have several times been inoculated with ascospores and conidia from *P. avium* but no infections have ever been obtained from such inoculations. Likewise no infection has ever been produced on *P. avium* by either ascospores or conidia from *P. virginiana* or *P. serotina.*

During the last weeks of April of this year a large series of cross inoculations were made in another greenhouse on all the different species before mentioned; but these resulted in almost total failure, probably because of the low temperature of the house. No infections were obtained except with the organism from *P. virginiana* and *P. serotina.* Only the Cylindrosporium conidia from *P. virginiana* were used and no infections were obtained except on *P. virginiana* where infection was rather abundant. Both ascospores and conidia of *P. serotina* were used. From the conidial inoculations infections occurred on *P. serotina* only. The ascospores however produced infection on *P. serotina, P. virginiana* and *P. mahaleb.*

The leaves of *P. virginiana* have also been infected with a pure culture obtained from conidia of the Cylindrosporium on the fruits of the same species. This shows that one species is capable of infecting both leaves and fruit of this host.

SYSTEMATIC

The study of morphological and cultural characters of Cylindrosporium from the various host species showed some slight variations, but none which were prominent or constant enough to be of specific value. It was thought, therefore, before a comparison of the perfect stage from the different hosts was made, that there was but a single species with several more or less distinct forms on these hosts.

Relation of Species of Cylindrosporium to the Natural Subdivisions of Prunus.—A careful study of the ascogenous stage from the different hosts showed that there were marked and constant differences by which the forms might be divided into three distinct groups. The first group includes all those which have the type of development found on *P. avium* (in which the ascocarp extends from one epidermis of the leaf to the other in contrast with those in which the ascocarp is only subepidermal). They are distinct in the shape of the ascocarp, its position in the leaf tissue, and in the shape and size of the asci.

Those having the subepidermal ascocarps fall into two distinct groups. On the plums (*P. domestica* and *P. insititia*) the fruit bodies are coal black with a very decided tendency to be aggregated in clusters, the asci are smaller, more slender than in the other forms, and are almost filled by the spores which are more slender than in either of the other forms.

Contrasted with this we find on *P. virginiana*, *P. serotina*, and *P. mahaleb* the light (yellowish brown to dull orange) colored fruit bodies, larger asci, and apothecial conidia much larger than in the other forms. The covering of the fruit body is composed of more delicate, thinner-walled cells.

After this grouping was made it was found that the hosts of each group fall in the same natural group of the genus Prunus, with the possible exception of *P. mahaleb.* The hosts of the first group are all in the subgenus Cerasus of Engler and Prantl, with flowers in umbels. Those of the second group are plums which are placed in the subgenus Prunophora by the same authors, while those of the third, with the exception of *P. mahaleb*, are placed by them in the subgenus Padus which has the flowers in elongated racemes. *P. mahaleb* has its flowers in a short raceme, and is placed by them in the subgenus Cerasus but in a group separate from the other members of the subgenus. Britton places it in a group with *P. virginiana* and *P. serotina* all having flower clusters terminating branches of the present year's growth.

The results of cross inoculations show a correlation of hosts with characters of the ascogenous stage. In no case has it been found possible to transfer, by inoculations, the fungus from hosts of one group to hosts of another group.

The fact that the hymenium of the ascocarp is surrounded—until nearly mature—by a wall of tissue which finally opens in a stellate manner places all forms of the fungus in the Phacidiales. The relation of the ascocarps to the leaf tissue (covered by the adherent host epidermis), the shape of the asci, and the elongated colorless spores grouped in a fascicle show a distinct relation to Coccomyces in which genus therefore all forms are included. Had only the light-colored form on the Padus group been found there might have been some question as to its relation to this genus, since Coccomyces is said to have black ascocarps; but the fact that three forms, so evidently related as those under consideration, show such wide variation in color indicates that the color of the ascocarp is here not a character of generic rank. Because, however, of the morphological and biological differences, before mentioned, the forms are divided into three species, as follows: *Coccomyces hiemalis* to include the forms on *Prunus avium, P. cerasus*, and *P. pennsylvanica; Coccomyces prunophorae* n. sp. to include the forms on the plums (*P. americana, P. domestica* and *P. insititia*); and *Coccomyces lutescens* n. sp. to include the forms on *P. serotina, P. virginiana*, and *P. mahaleb.* For these species the following characterization is given:

DESCRIPTION OF SPECIES

Coccomyces hiemalis Higgins, Science, N. S. **37**: 637 and 638. 1913. Ascocarps embedded in the tissue of the leaf—usually filling the entire space between the lower and the upper epidermis—both of which usually adhere to the wall of the ascocarp, scattered to subaggregate, ovate to orbicular, dark brown or black, at first closed but at maturity opening by irregular stellate slits on the under side of the leaves, 125–210μ in diameter; hymenium pale gray to flesh-colored; asci clavate with a long stout pedicellate base, and abruptly papillate apex, 8-spored, 70–95 × 11–14μ; paraphyses filiform, septate, apex slightly enlarged, often hooked, often forked; ascospores linear, 33–50 × 3.5–4.5μ (the smaller size given in original description was due to a typographical error), continuous or 1–2 septate, fascicled in large end of the ascus; apothecial conidia produced on short conidiophores in apothecia after shedding of ascospores, long, slender, 50–80 × 2.5–4μ, curved, continuous or 1–2 septate.

Conidial stage (*Cylindrosporium hiemalis*): Mycelium intercellular with small haustoria which penetrate the host cells; spots small, brown or reddish brown, sometimes dropping out and producing "shot holes"; acervuli amphigenous or more commonly hypophyllous, subepidermal, finally erumpent exposing the spores; conidia elongate, curved or flexuous, 45–65 × 2.5–4μ, continuous or 1–2 septate; microconidia (spermatia?) produced in same acervulus in late summer and fall, small, continuous 4–5 × 1.5μ.

Conidial stage parasitic in leaves of *P. avium, P. cerasus*, and *P. pennsylvanica.* Ascigerous stage saprophytic, appearing the last of April to June, on fallen leaves of the same hosts following the conidial stage.

Latin diagnosis: Ascomatibus hypophyllis sparsis interdum subaggregatis, innatis, punctiformibus, fuscis vel nigris, ovatis vel orbicularibus, 125–250μ lat., primum clausis, deinde in lacinias plures acutas dehiscentibus; disco pallido carneo vel grisea; ascis clavatis crassiuscule stipitatis, 70–90 × 11–14μ, octosporis, apice papillato; paraphysibus filiformibus, simplicibus aut ramosis, apice recto aut curvato; sporidiis fasciculatis, linearibus 35–50 × 3.5–4.5μ, simplicibus aut 1–3 septatis; conidiis in apotheciis filiformibus, flexuosis, 50–80 × 2.5–4μ, 1–2 septatis.

Hab. In dejectis foliis Prunii avii, P. cerasi, et P. pennsylvanicae.

Status conidicus: maculis sparsis vel confluentibus, minutis, brunneis aut rufis-brunneis, interdum majusculis dejectis, acervulis solitariis amphigenis, subepidermicis, disciformibus; conidiis filiformibus, flexuosis, denique emergenti-superficialibus, hyalinis 45–65 × 2.5–4μ, simplicibus aut 1–2 septatis; conidiis minoribus autumno, hyalinis, continuis 4–5 × 1.5μ.

Hab. In foliis vivis Prunii, P. cerasi, et P. pennsylvanicae.

Coccomyces prunophorae n. sp. Ascocarps hypophyllous, usually aggregated, subepidermal, erumpent, disk-shaped to subglobose, 125–250 × 100–160μ, black, at first closed but at maturity opening in a stellate manner; hymenium light gray; asci clavate to clyindrical-clavate, 63–87 × 9–12μ, opening by a pore at the papillate tip; spores slender, straight, curved near the end, 40–60 × 2.5–3.5μ, at maturity almost filling the asci, continuous or 1–3 septate; paraphyses simple or branched, enlarged at apex, septate, about as long as asci; apothecial conidia produced on short conidiophores inside the apothecia, following the asci, usually once septate, 40–60 × 2.5–3.5μ, resembling ascospores but usually stouter.

Conidial stage (*Cylindrosporium prunophorae*): mycelium intercellular with haustoria which enter host cells; spots small, brown or reddish brown, sometimes dropping out, producing "shot holes." Acervuli subepidermal, amphigenous, finally breaking through exposing the white mass of spores, conidia elongate, slender, straight or curved, 46–65 × 3.5–5μ, usually 1-septate; microconidia (spermatia?) found in same acervuli in late summer, small, continuous 4–5 × 1.5μ.

Conidial stage parasitic in leaves of plums (*P. domestica*, *P. insititia*, *P. americana*[7] and probably *P. spinosa*). Ascigerous stage saprophytic on fallen leaves of the same hosts, May to June, following the conidial stage of the previous summer.

Latin diagnosis: Ascomatibus hypophyllis, aggregatis vel sparsis, subepidermicis, erumpentibus, disciformibus vel subglobosis, 125–250 × 100–160μ, nigris, primum clausis deinde in lacinias plures acutas dehiscentibus; disco pallido griseo; ascis clavatis vel cylindraceoclavatis, fere sporidiis completis, 63–87 × 9–12μ, octosporis, apice papillato; paraphysibus filiformibus, simplicibus aut ramosis, septatis; sporidiis fasciculatis, linearibus 40–60 × 2.5–3.5μ aut 1–3 septatis; paraphysibus simplicibus aut continuis ramosis; sporidiis in apotheciis linearibus rectis vel flexuosis, 40–60 × 2.5–3.5μ, 1 septatis.

Hab. in dejectis foliis Prunii domesticae, et P. instititiae.

Status conidicus; maculis sparsis, vel confluentibus, brunneis aut rufis-brunneis interdum majusculis dejectis; acervulis solitariis, amphigenis, subepidermicis, disciformibus, conidiis filiformibus, rectis vel flexuosis, denique emergenti-superficialibus, hyalinis, 46–65 × 3.5–5μ, 1 septatis, conidiis minoribus autumno hyalinis continuis, 4–5 × 1.5μ.

Hab. In foliis vivis Prunii domesticae, P. insititiae, P. spinosae, et P. americanae.

Coccomyces lutescens n. sp. Ascocarps hypophyllous, subepidermal, erumpent, disk-shaped to flattened elliptical, 130–300 × 70–150μ, often depressed above before maturity, lutescent or dull orange in color, at first closed, then opening in an irregularly stellate manner, hymenium grayish to creamy white; asci clavate 70–80 × 14–19μ, opening by a pore in the papillate apex; spores elongated, fascicled in end of ascus, 40–51 × 3.5–4.5μ, continuous or occasionally once or twice septate; paraphyses usually simple though occasionally branched, slightly enlarged at tip, about as long as asci; apothecial conidia very long, 58–87 × 3.5–5μ, once-septate with usually a single vacuole in each cell.

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[7] Since infection and the Cylindrosporium acervulus can be produced on *P. americana* by the fungus from *P. insititia*, it is very likely that the perfect stage also develops on this species.

Conidial stage (*Cylindrosporium lutescens*): spots small, brown to reddish brown, in early summer dropping out from leaves of some species and producing rounded "shot holes"; mycelium intercellular, with haustoria which penetrate the host cells; acervuli subepidermal, amphigenous; conidia exuding in masses or in long tendrils from the break in the host epidermis, long, slender 50–87 × 3.5–5μ, continuous or 1–3 septate, microconidia (spermatia?) produced in the same acervuli in late summer, small, continuous, 4–5 × 1μ.

Conidial stage parasitic in leaves of *P. serotina* and in leaves and fruits of *P. virginiana*, and *P. mahaleb*. Ascigerous stage saprophytic on fallen leaves of the same species in May and June following the conidial stage of the previous summer.

Latin diagnosis: Ascomatibus hypophyllis, sparsis, subepidermicis, erumpentibus, disciformibus, 130–300 × 70–150μ, luteis vel ferrugineis, primum clausis deinde in lacinias plures acutas dehiscentibus; disco pallido carneo vel griseo; ascis clavatis, crassiuscule stipitatis, 70–80 × 14–19μ, octosporis, apice papillato; paraphysibus, filiformibus, simplicibus aut ramosis; sporidiis fasciculatis, linearibus, 35–50 × 3.5–4.5μ, simplicibus aut 1–3 septatis; conidiis in apotheciis filiformibus, flexuosis, 50–80 × 2.5–4μ, 1-septatis.

Hab. in dejectis foliis Pruni serotinae, P. virginianae et P. mahalebis.

Status conidicus; maculis sparsis vel confluentibus minutis brunneis aut rufisbrunneis, interdum majusculis dejectis; acervulis solitariis, amphigenis, subepidermicis, disciformibus; conidiis, filiformibus, flexuosis, denique emergenti-superficialibus, hyalinis, continuis 50–87 × 3.4–5μ, 1 septatis; conidiis minoribus autumno hyalinis, continuis 4–5 × 1.5.

Hab. in foliis vivis Prunii serotinae, P. virginianae, et P. mahalebis.

General Discussion

There are many points of interest in the life history and development of the Cylindrosporium on stone fruits, not the least of which is their relation to the host tissue. The parasitic stages of Ascomycetes, other than the Perisporiales which have epiphytic mycelium, usually kill the host tissue outright. In two notable exceptions to this, *Gnomonia erythrostoma* Pers., and *Polystigma rubrum* (Pers.) D. C. no haustoria have been found. Haustoria have been described for a large number of the Perisporiales (in the Erysiphaceae by many workers and by Maire (18) for Meliola[8] and Asterina). In one of the Erysiphaceae, *Oidiopsis taurica* Salmon, the mycelium is endophytic, and it is partly so in *Phyllactinia corylea* (see Palla (21) Smith (25)

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[8] Meliola is placed in the Perisporiales by Saccardo.

Salmon (24)); but even here the host tissue is not killed. In this respect Cylindrosporium resembles the last named species, and the presence of haustoria here is probably related to this behavior.

The formation of spermatia-like bodies in connection with structures which appear to be homologous with the ascogonial branches of the Collemaceae is also very interesting. The function of these bodies is not known. Several attempts to germinate them have always resulted in failure, but considering the difficulty of germinating the normal conidia this is not a very strong argument in favor of their spermatial nature. Their position on the surface of the stroma with the trichogyne of the ascogonium (?) projecting among them would certainly facilitate fertilization, if they do now function or have ever functioned as sexual organs. The most nearly similar arrangement is found in species of Physma. Here there are several ascogonial branches arising in the base of the spermogonium, as in Coccomyces on stone fruits, but passing around the spermogonium to the surface of the thallus. See Stahl (27), Sturgis (28), and others.

The ascogonial structures found in *Gnomonia erythrostoma* have been studied by Brooks (6) and were not found to function as female sexual organs. On the contrary he thinks the ascogenous hyphae arise from vegetative cells. A similar condition was found in *Polystigma rubrum* by Blackman and Wellsford (5). From analogy and from the fact that the ascogonial structures appear to disintegrate in many cases, one is led to think that in Coccomyces also the ascogonium does not function as a sexual organ. However the fact that the ascogenous hyphae arise from several points similarly situated in the ascocarp indicates that at least a part of the structure may function as an ascogonium.

CONTROL MEASURES

Now that the complete life history of the fungus is known, it should be much easier to devise methods for controlling the disease. Since it lives over winter in the dead leaves it is very important that leaves from trees which are infested with the disease be raked together and burned or buried. If all such diseased leaves are destroyed in and near the cherry orchard there is little danger of the disease appearing the following season in such abundance as to be of a serious nature.

If for any reason destruction of the diseased leaves is not feasible or desirable and one must depend on sprays to keep the disease in check, the spraying should begin early, at least by the middle of May.

The only common wild species in this region which may harbor the fungus which attacks the sweet and sour cherries is *P. pennsylvanica*.

There appears to be no danger of the disease passing from the wild choke cherry or the wild black cherry (*P. serotina*) to the sweet or sour cherry; but the fungus from the former may infect nursery seedlings of *P. mahaleb*.

The fungus on the plums has so far been found to infect nothing but plums, but some of the wild plums, certainly *P. americana*, may harbor the fungus.

CONCLUSIONS

1. There are at least three species of Cylindrosporium parasitic on species of the genus Prunus, which in their conidial stage resemble each other very closely. Whether or not *Cylindrosporium padi* Karst. also occurs in North America is not known; but from the fact that it occurs on *P. padus*, which has racemose flower clusters similar to *P. serotina* and *P. virginiana*, it is perhaps the same as that occurring on the two last named species.

2. The conidial stroma of Cylindrosporium on Prunus develops centrifugally and is never turned up at the edges so as to resemble a pycnidial structure.

3. The mycelium of the species of Cylindrosporium studied is intercellular and obtains its food, in part at least, by means of haustoria which penetrate the host cells. Very often a cellulose sheath is then deposited around the haustorium by the host protoplasm. It seems that no toxin or substance injurious to the host protoplasm is secreted by the fungus, but a few of the host cells are killed probably by drying.

4. "Shot hole" formation in the leaves of the host is apparently correlated with the presence of amygdalin. The amygdalin molecule breaks down into simpler molecules thereby probably increasing the osmotic pressure which causes the cells surrounding the spot to enlarge forming the separation layer.

5. Beside the Cylindrosporium conidia three other spore forms are found in the life cycle of the species studied, viz.: microconidia (spermatia-like bodies), ascospores, and apothecial conidia. All of these except the microconidia are known to propagate the fungus on living leaves.

6. The microconidia are not produced in pycnidial structures, as reported by Arthur (4) and by Pammel (22), but on the surface of the conidial stroma.

7. While the microconidia (spermatia?) are being formed on the surface of the stroma, ascogonia-like structures are formed with their free end (trichogyne?) projecting above the surface. If these structures do now function or have ever functioned as sexual organs, their formation at the same time and on the same stroma would make fertilization more certain.

8. The fungus passes the winter as a stroma-like body in the fallen leaves, which in the early spring develops into an apothecium of the Phacidiaceous type.

9. That the ascocarps are genetically connected with Cylindrosporium has been shown by their continuous development from the stromata of Cylindrosporium, and by producing infection and Cylindrosporium acervuli in living leaves when inoculated with ascospores from leaves, or with conidia from pure cultures from these ascospores.

10. The forms of Coccomyces found on the eight species of Prunus fall naturally into three species, one on each of three more or less natural subdivisions of the host genus.

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DESCRIPTION OF PLATES XIII–XIV

PLATE XIII

FIG. 1. Photograph of leaf of Prunus avium with ascocarps, magnified about 2 diameters.

FIG. 2. Photograph of a portion of same leaf showing ascocarps opening by stellate slits, magnified about 6 diameters.

In the following three plates the figures are from camera lucida drawings and the magnification is indicated after each figure.

PLATE XIV

FIG. 3. Section of leaf of Prunus avium showing the subepidermal acervulus bearing microconidia (spermatia ?) while still covered with macroconidia of Cylindrosporium. × 250.

FIG. 4. Pycnidium of Septoria pruni Ellis in leaf of Prunus americana, from "North American Fungi" II, 1151. × 1,300.

FIG. 5. Section of leaf of Prunus serotina showing subepidermal conidia bearing acervulus of Septoria cerasina Peck (= Cylindrosporium), from type material. × 250.

FIG. 6. Ascocarp of Coccomyces hiemalis in leaf of Prunus avium, showing remains of epidermal cells above and below and lignified cells of host vascular bundle near center of ascocarp. × 250.

FIG. 7. Ascocarp of Coccomyces prunophorae. × 250.

FIG. 8. Young ascocarp of Coccomyces hiemalis in leaf of Prunus avium showing portions of three ascogonial (?) coils and two trichogynes. The pseudoparencyhmatous covering of the ascocarp is beginning to form and conidiophores (microconidiophores) are beginning to disintegrate above the center of the young fruit body. × 250.

FIG. 9. Coiled ascogonial (?) branch from a slightly later stage in which the trichogyne is disintegrating.

FIG. 10. Young asci and portions of ascogenous hyphae from a section of developing ascocarp of Coccomyces hiemalis.

FIG. 11. Old ascocarp of Coccomyces hiemalis producing "apothecial conidia." × 250.

PLATE XV

FIG. 12. Section of leaf of Prunus virginiana showing entrance of germ tube of Cylindrosporium spore through a stoma and subsequent growth of mycelium. From artificial infection under bell jar four days after inoculation. × 450.

FIG. 13. Early stage in the development of stroma and acervulus of Cylindrosporium beneath the upper epidermis of same host, five days after inoculation. Two haustoria have entered an epidermal cell. × 450.

FIG. 14. Section of leaf of Prunus virginiana showing amphigenous nature of Cylindrosporium acervuli and the separation of the diseased spot. × 47.

FIG. 15. Portion of section similar to fig. 14 more highly magnified showing the enlarged cells of separation layer and walls of ruptured cells. × 450.

FIG. 16. Later stage, showing the suberized layer (the cell walls of which are represented by heavier lines) and the remains of the enlarged cells now dead. × 450.

FIG. 17. Young haustoria, showing nuclei and vacuoles, in cells of bundle sheath in leaves of Prunus virginiana.

FIG. 18. Haustoria in mesophyll cell of the same leaf surrounded by cellulose sheaths.

FIG. 19. Developing ascocarp of Coccomyces lutescens in leaf of P. virginiana, showing the subepidermal position with remains of the gelatinized conidiophores and the early appearance of the paraphyses. × 250.

FIG. 20. Mature ascocarp of same with asci and paraphyses. × 250.

FIG. 21. Developing ascocarp of Coccomyces lutescens on Prunus serotina still covered by host epidermis. × 250.

FIG. 22. Mature ascocarp of same. × 250.

PLATE XVI

FIG. 23. Asci and single paraphysis of Coccomyces hiemalis from Prunus avium. × 450.

FIG. 24. Ascospores of same more highly magnified. × 625.

FIG. 25. Ascospores of same germinating in an agar culture five days old.

FIG. 26. Asci and paraphyses of Coccomyces hiemalis from Prunus pennsylvanica, spores are mature in one on left. × 450.

FIG. 27. Conidia (Cylindrosporium) of Coccomyces hiemalis on Prunus pennsylvanica. × 450.

FIG. 28. Asci and paraphysis of Coccomyces hiemalis on Prunus cerasus. The spores have been shed from one of the asci, and are scarcely mature in other. × 450.

FIG. 29. Germinating conidia (Cylindrosporium) of Coccomyces hiemalis. × 450.

FIG. 30. Microconidia (spermatia ?) and microconidiophores of Coccomyces hiemalis from P. avium. × 450.

FIG. 31. Apothecial conidia of Coccomyces hiemalis on P. avium. × 450.

FIG. 32. Same germinating after two days in water culture.

FIG. 33. Asci and paraphyses of Coccomyces prunophorae from P. domestica. × 450.

FIG. 34. Ascospores from same more highly magnified. × 650.

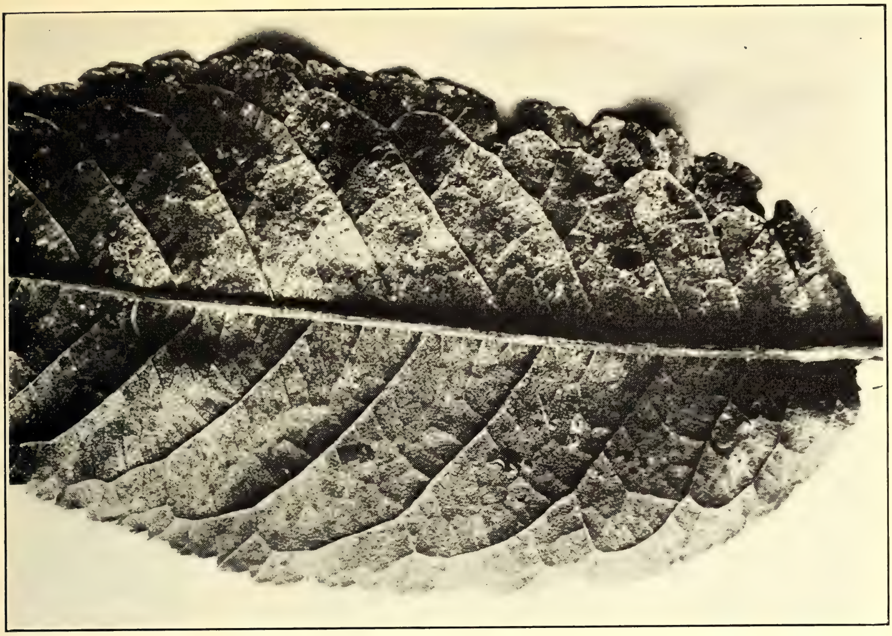
FIG. 35. Apothecial conidia of Coccomyces prunophorae. × 450.

FIG. 36. Conidia (Cylindrosporium) of Coccomyces prunophorae. × 450.

FIG. 37. Ascus of Coccomyces lutescens from Prunus mahaleb.

FIG. 38. Ascospores of same more highly magnified. × 625.

FIG. 39. Microconidia and microconidiophores of Coccomyces lutescens. × 450.



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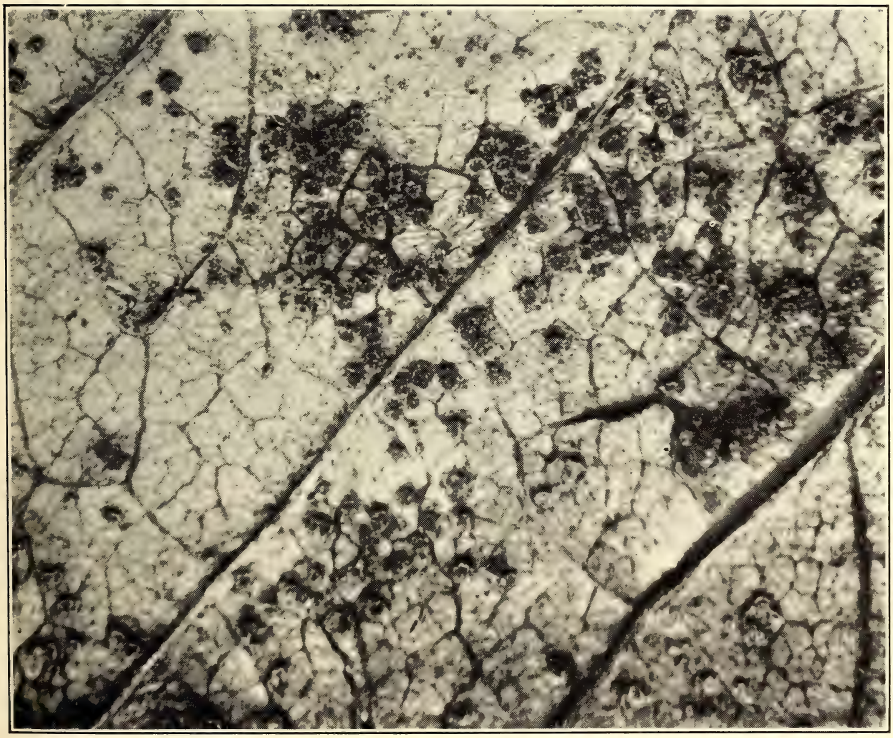

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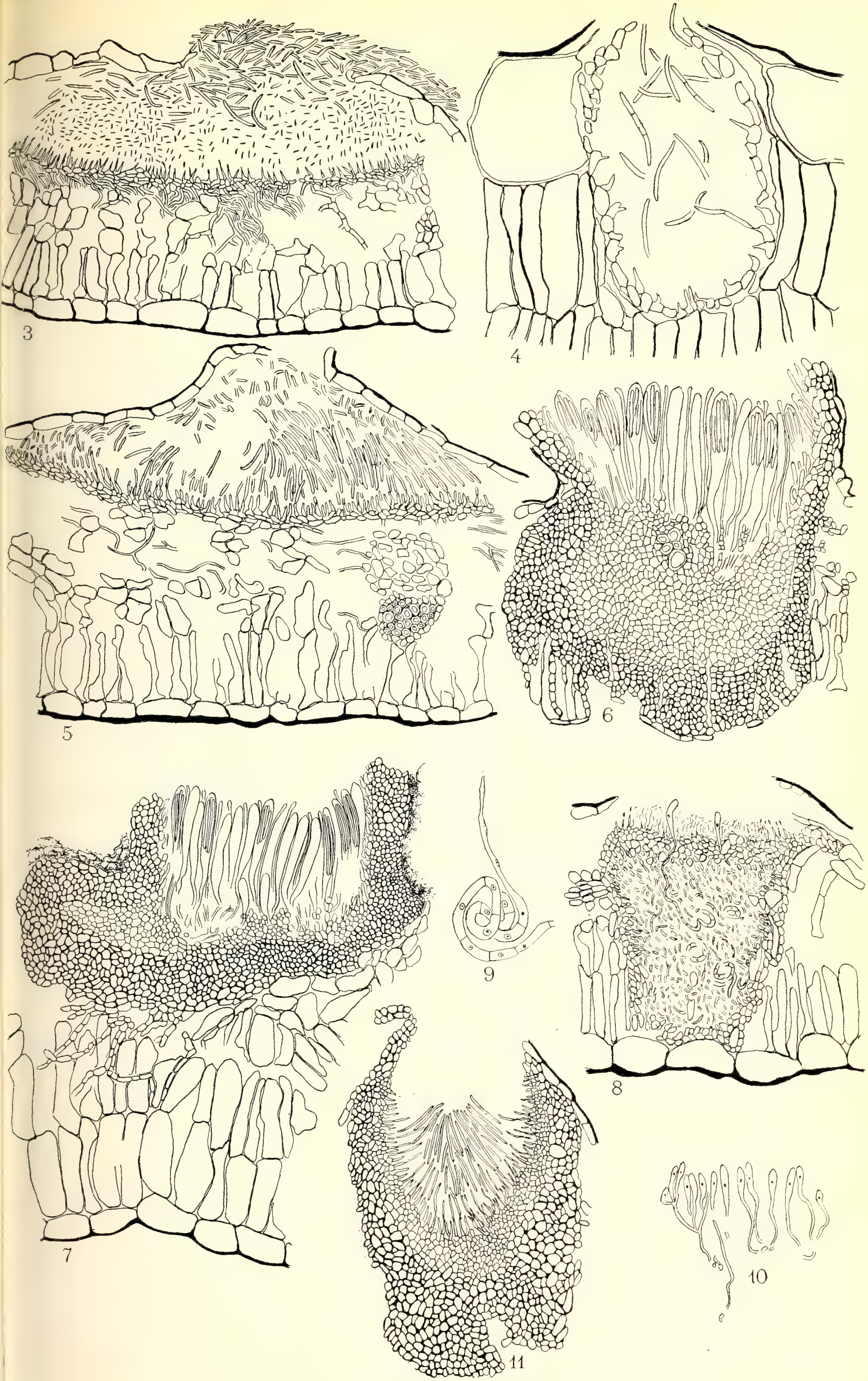
Higgins: Life History of Cylindrosporium.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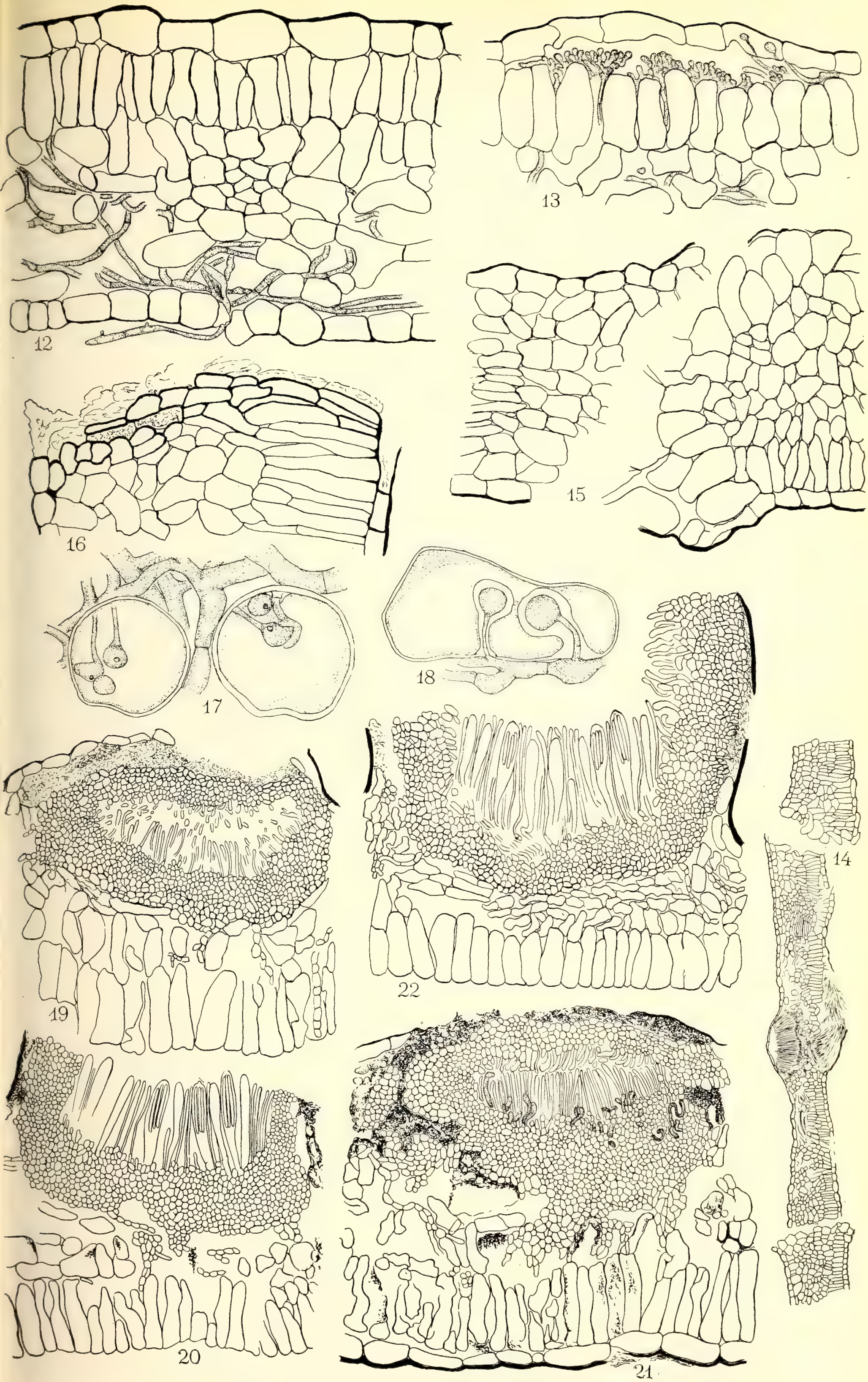
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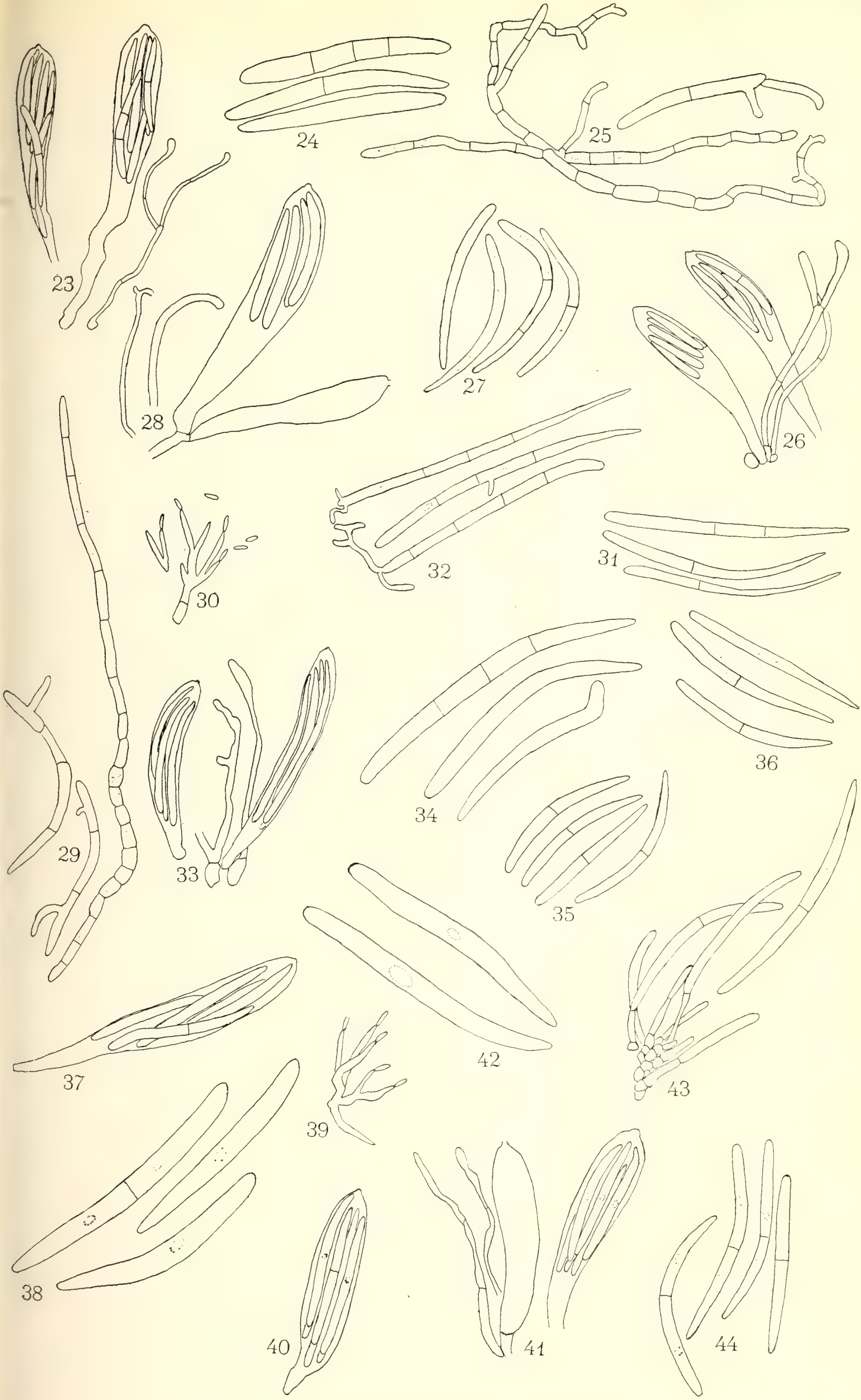
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HIGGINS: LIFE HISTORY OF CYLINDROSPORIUM.

FIG. 40. Ascus of Coccomyces lutescens from P. virginiana.

FIG. 41. Asci and paraphyses of Coccomyces lutescens from Prunus serotina. × 450.

FIG. 42. Ascospores of same more highly magnified. × 625.

FIG. 43. Apothecial conidia and conidiophores of Coccomyces lutescens on Prunus serotina.

FIG. 44. Conidia (Cylindrosporium) of Coccomyces lutescens on Prunus serotina.

THE PHYSIOLOGICAL WATER REQUIREMENT AND THE GROWTH OF PLANTS IN GLYCOCOLL SOLUTIONS[1]

Alfred Dachnowski and R. Gormley

The relation of plants to peat and humus soils has been of the utmost importance to students of plant physiology because of the nature of the metabolism involved. It is of equally great interest to ecologists in so far as the native social vegetation units covering an organic soil area take on a characteristic spacial distribution—a mutual exclusiveness—and yet gradually intrude upon and replace one another in a fairly genetically related succession in time.

The contributions of the senior author (5–8) have been mainly with a view toward an analysis of the several habitat factors, and a study of the organic constituents of peat soils in relation to plant activity. Historical accounts and a number of experimental data on the causal and limiting factors of a bacterial, chemical, and other nature have been brought together in Bulletin 16, Geological Survey of Ohio, 1912 (7).

The present paper, though only of a preliminary nature, is a continuation of the physiological studies and has been made in the hope that it will draw more pointed attention to the metabolic processes of a number of plants, some of which do, while others do not, seem to be able to extend their range of distribution beyond a typical habitat. In addition, the attempt is made with a view to understanding more clearly the nature of the "association factor" which seems of great importance in determining the geographical distribution of the higher social vegetation units, and the edaphic "preferences" of plants more or less nomadic in their tendency.[2]

The principal organic compounds from which peat and humus are derived are the various lignocelluloses, the carbohydrates, proteins and other substances produced in the course of the growth and reproduction of bog and swamp vegetation. During the decomposition of these by weathering and biochemical changes, by bacteria and fungi, various other compounds and transition products arise. The definite recognition of a number of these constituents from mineral soils has led to a fuller understanding of the chemistry of organic soils (16), but for many of the peat and humus compounds the names and written reactions have little meaning as now understood; the organic degradation products are of great complexity and they cannot as yet be easily isolated and identified. It is well known, however, that during a gradual hydrolysis of proteins, for example, there arise chiefly diamino and monoamino acids. Glycocoll, $H_2C.NH_2.COOH$, is one of the simpler degradation products and has been derived from the tissue and the seeds of a number of plants.

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[1] Contribution from the Botanical Laboratory of Ohio State University, No. 82.

[2] A more explicit statement will appear shortly dealing with the association factor and the evolution of social vegetation types, based upon observations which one of the authors obtained during the recent International Phytogeographic Excursion in America.

Important problems of agriculture and economic considerations are involved in the ability to accomplish the growth of crop-yielding plants within a shortened life cycle and at lessened energy requirements and expense. Hence the question of the efficiency of a plant in utilizing directly nitrogenous compounds other than nitrates and ammonia has interested a number of investigators, both from the standpoint of the problems of soil fertility and that of plant metabolism and enzyme action. Hansteen (11), Molliard (13), Schreiner (15), Hutchison and Miller (12), and Borowikow (1) have ascertained the effects of water solutions of glycocoll on plants. They report, more or less uniformly, that the effects of glycocoll are beneficial, and that a gain results of nitrogenous matter in the plants. Hansteen found that *Lemna* in the absence of light produces proteins, but that the effect on growth is slightly harmful. Molliard obtained better results with radishes. Schreiner found that glycocoll is beneficial to wheat seedlings in solutions containing 0.1 per cent and less of the solute. Hutchison and Miller working with peas report inconclusive results; the nitrogen content of one of the pea-cultures was increased, but a slight decrease was noticed in another culture. The more recent experiments of Borowikow with *Helianthus* indicate that the solution retards growth.

It is not known to what extent amino-acids occur in peat soils. Although we are unable to extract at present any beneficial nutritive materials from them by ordinary chemical means, it is quite likely that many of the bacteria and fungi are able to do so by excreting enzymes with dissolving capacities. The question possesses additional interest in the case of heaths and other bog plants having mycorrhiza, since we have no knowledge of the form of organic peat constituents which they can assimilate.

In the continuation of this work the same general methods described in previous publications have been used. For the glycocoll employed in these experiments we are indebted to Prof. W. McPherson, of the department of chemistry of this University, whose interest in this problem has been voiced in his vice-presidential address before section C of the American Association for the Advancement of Science (10). Grammolecular solutions of varying concentrations were prepared in distilled water which had been filtered through carbon (lamp black). The experiments were begun in October, 1913. The bog plants used in these tests were obtained, in November, 1913, from the cranberry-sphagnum association on Cranberry Island at Buckeye Lake, Ohio.[3] With the exception of wheat, these and all other plants used were cuttings of known green weight. The plants were freed from dead material, washed repeatedly in distilled water, fastened to sterilized perforated corks by means of absorbent cotton, and transferred to sterilized, wide-mouthed, paper-covered glass jars of 500 cc. capacity. No other precautions were taken against the presence of nitrifying or other organisms. Although sterilization was imperfectly maintained, the results obtained with the selected series are fairly satisfactory. Each experiment was continued for 7 to 14 days, according to the amount of water transpired before renewing the culture solution. The different cultures always stood side by side in the university greenhouse. A record was kept of the weight of water absorbed and the quantity transpired. The difference between the initial and final green weight of the plants was taken to represent the amount of growth.

The original intention, to show what quantities of glycocoll are assimilated directly and thus increase the dry weight of plants, could not be adhered to in this series of experiments. Arrangements have been completed whereby the biochemical investigations of the problem will be carried out as soon as the improvements in the equipment of the new laboratory devoted to this work have been provided. For the present only a few of the preliminary experiments are here grouped according to the response of the plants and indicated by data on transpiration, amount of water absorbed and retained, and the gain or loss in the weight of plants. Related data from experiments with cuttings of tomato, Eupatorium sp., and various cereals in nutrient solutions are omitted; also the results with plants cultivated under humid and arid atmospheric conditions, and, with such, the growth and stem elongation of which terminates with the formation of flowers and seeds. In the main the data correspond with the conclusions drawn from the experiments submitted in Tables I to IX, and show that transpiration is not directly correlated with and hence not a measure of growth.

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[3] Geol. Sur. Ohio Bull. 16: 239.

The chief results of the experiments given below may be described as follows: The plants grown in the glycocoll solutions show an increase in green weight above that of the quantity of water absorbed from the solution and retained within the tissues. The predominant constituent of this gain is in part undoubtedly the glycocoll absorbed and assimilated, and to a lesser degree due to the photosynthesis of carbohydrates.

To what extent irregularities of growth tend to be followed by compensatory processes is not known. The loss in weight of plants indicated in Tables I and VII does not express a more or less prolonged rate of transpiration in excess of the rate of absorption for the same period. No loss of turgor and no form of wilting, temporary or permanent, is here involved (2, 4, 9). The deficit is in the removal of reserve materials from the tissues, and is unaffected by the external set of atmospheric conditions under which the plants were transpiring. The apparently inevitable conclusion is entertained that the problem of the water requirement of plants and the criteria for the wilting coefficient, in particular the relation between the water content of the plant and that of the soil at the time of wilting, need to be investigated more quantitatively than has been heretofore attempted. Investigations are now in progress.

The plants in solutions 2, 3, 4 (Table IV), 5 (Table V), and 3 (Table VII), retained their weight but changes were going on in the body proportions of roots. It is obvious that the constancy of the green weight of plants is therefore not an indication of lack of growth, *i. e.*, growth increments are not the only criterion or the only measure of growth. No estimate can be offered as yet of the correlation between weight and size of plant under these conditions. The suppression of growth is not primarily attributable to energy requirements, as is seen in Table VII. The failure to promote growth may be due to the lack of variety and of a more balanced condition of materials for development, *i. e.*, it may be due to the inefficiency of isolated food constituents, such as glycocoll, to supply material for tissue construction. The retention of water is smaller in plants having very low food reserves, and becomes steadily less as the glycocoll content of the solution becomes decreased or the time element in the renewal of the culture solution is extended. The progressive decline in weight is obviously a pathological manifestation. Experiments are now in progress to determine also to what extent the age of plants, their resting period, and the upper and lower limit of transpiration, which plants of any species rarely exceed, may modify the percentage of water retained. Many problems regarding the absorption of CO_2 under these conditions are still in need of future investigation, especially since the importance of the atmospheric CO_2 gradient gains an unsuspected prominence in the supposition that xeromorphy of ancient and modern vegetation may be due in part to a modified gas interchange.[4]

To what extent the numerical relation between the quantity of water retained and the amount of growth provides a value which may serve as a criterion for life zones, yields of crops, and related problems of physiology and ecology remains to be determined. The present paper is intended to throw some light on the more fundamental problem of the "water requirement" (3) of plants during growth and metabolism. By way of comparison the essential data of investigations on the acid tolerance of these plants have been given in tabular form, to enable the reader to draw further conclusions on the acidity problem of peat soils.

No special discussion is required to consider the results, here presented, in detail. An examination and comparison of the data will lead to the following conclusions:

1. The value of the transpirational water loss in the experiments cited is a function of the vapor pressure of water affected by the quantity of the salts in solution, and the factors modifying the atmospheric conditions.

2. The transpiration value of plants, when correlated with physical conditions of soil solution and atmosphere influencing it, and when expressed as a ratio in terms of these or any other factor affecting it directly, should be called the *ecological* water requirement. As such it is sufficiently distinctive to characterize diverse plants and diverse habitats, and to indicate the limiting conditions and the range of deviation of the water relation of vegetation.

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[4] Geol. Sur. Ohio Bull. 16: 277.

3. The process of absorption of glycocoll is not connected with the transpirational water loss, but with the differential permeability of the absorbing root cells, with the efficiency of the nutritive metabolism characteristic of the plant and the amount of water retained within the plants.

TABLE I

TRANSPIRATION AND GROWTH OF *Scheuchzeria palustris* IN GLYCOCOLL SOLUTIONS
December 17, 1913, to January 17, 1914

Culture Solution	Amount of water in grams			Gain or loss in weight of plants (in grams)
	Absorbed	Transpired	Retained	
1. Distilled water	6.75	6.50	0.25	
	6.55	6.20	0.35	0.60
2. *n*/800 glycocoll	6.00	5.30	0.70	
	5.05	4.50	0.55	1.40
3. *n*/1,600 glycocoll	6.15	5.90	0.25	
	4.65	4.20	0.45	0.80
4. *n*/3,200 glycocoll	7.80	7.00	0.80	
	5.28	5.20	0.08	0.95
5. *n*/6,400 glycocoll	10.25	9.50	0.75	
	8.55	8.20	−0.15	0.85
6. *n*/12,800 glycocoll	12.30	11.90	0.40	
	7.00	7.10	−0.10	0.40

Rhizomes in solutions 2–6 produced normal roots and roothairs. Best growth of roots in *n*/6,400 solution.

Atmometer, 443 cc. Temperature, 8°–31° C. Rel. humidity, 43%–100%.
Barometer, 28.85–29.95 cm.

TABLE II

TRANSPIRATION AND GROWTH OF *Scheuchzeria palustris* in HCl SOLUTIONS
November 23, to December 17, 1913

Culture solution	Amount of water in grams			Gain or loss in weight of plants (in grams)
	Absorbed	Transpired	Retained	
1. Bog water	4.00	3.60	0.40	0.40+
2. *n*/400 HCl	9.75	9.25	0.50	0.50
3. *n*/800 HCl	7.05	6.40	0.65	0.65
4. *n*/1,600 HCl	8.05	7.65	0.40	0.40
5. *n*/3,200 HCl	4.45	3.95	0.50	0.50
6. *n*/6,400 HCl	3.65	3.20	0.45	0.45
7. *n*/12,800 HCl	5.70	5.20	0.50	0.50

Plants in bog water with roots and long roothairs. Growth of roots retarded in strong acid solutions. Roots brownish and slightly gelatinized in weaker acid solutions; roothairs occasional. Atmospheric conditions as in Table VIII.

4. The insufficiency of a salt operates as a limiting factor to growth but transpiration does not decrease consistently with the retardation in growth.

5. The amount of water retained by plants is decreased when the strength of the solution is increased beyond a certain optimum concentration. The available water rather than the solute is then the

TABLE III

TRANSPIRATION AND GROWTH OF *Eriophorum virginicum* IN GLYCOCOLL SOLUTIONS
December 17, 1913, to January 17, 1914

Culture solution	Amount of water in grams			Gain or loss in weight of plants (in grams)
	Absorbed	Transpired	Retained	
1. Distilled water	7.00	6.80	0.20	
	8.50	8.10	0.40	0.60
2. *n*/800 glycocoll	10.10	9.30	0.80	
	11.60	11.30	0.30	1.30
3. *n*/1,600 glycocoll	10.55	9.80	0.75	
	19.55	18.40	1.15	1.98
4. *n*/3,200 glycocoll	11.85	11.50	0.35	
	14.55	13.90	0.65	1.20
5. *n*/6,400 glycocoll	11.80	11.00	0.80	
	15.20	14.10	1.10	1.95
6. *n*/12,800 glycocoll	9.60	8.80	0.80	
	25.40	24.70	0.70	1.65

New roots with roothairs near upper portion of rhizome, increasing in number in dilute solutions. Atmospheric conditions as in Table I.

TABLE IV

TRANSPIRATION AND GROWTH OF *Eriophorum virginicum* IN HCl SOLUTIONS
November 23 to December 17, 1913

Culture solution	Amount of water in grams			Gain or loss in weight of plants in grams)
	Absorbed	Transpired	Retained	
1. Bog water	4.05	3.70	0.35	0.35+
2. *n*/400 HCl	3.70	3.70	0.00	0.00
3. *n*/800 HCl	3.85	3.85	0.00	0.00
4. *n*/1,600 HCl	3.95	3.95	0.00	0.00
5. *n*/3,200 HCl	4.40	4.10	0.30	0.30
6. *n*/12,800 HCl	3.00	2.60	0.40	0.40

Plants with new roots and roothairs in bog water solutions, and in weak acids. Root growth absent in strong solutions. Absorbing surface beginning to increase in solution No. 4. Atmospheric conditions as in Table VIII.

limiting factor. Unlike plants react differently to physiologically limiting water conditions. The variations are inherent,—peculiarities of the growth capacity and the metabolism of plants.

6. Plants may show a loss in weight but not a corresponding loss in the amount of water transpired; on the other hand, increase in growth may take place with little or no change in transpiration.

7. Changes in body weight, if taken as the measure of growth, may be pronouncedly altered by the retention of water as well as by the deposition or removal of reserve materials in the tissues. The

TABLE V

TRANSPIRATION AND GROWTH OF *Vaccinium oxycoccus* IN GLYCOCOLL SOLUTIONS
December 17, 1913, to January 17, 1914

Culture solution	Amount of water in grams			Gain or loss in weight of plants (in grams)
	Absorbed	Transpired	Retained	
1. Distilled water	4.95	4.35	0.60	
	4.35	4.30	0.05	0.65
2. *n*/800 glycocoll	9.30	8.70	0.60	
	7.85	7.70	0.15	0.75+
3. *n*/3,200 glycocoll	7.25	7.05	0.20	
	9.80	9.20	0.60	0.80+
4. *n*/6,400 glycocoll	7.65	7.10	0.55	
	7.85	7.80	0.05	0.60+
5. *n*/12,800 glycocoll	5.30	4.90	0.40	
	6.30	6.30	0.00	0.40+

New roots with mycorrhiza and devoid of roothairs; short, dense clusters in weaker solutions. Root growth best in solution No. 5. Atmospheric conditions as in Table I.

TABLE VI

TRANSPIRATION AND GROWTH OF *Vaccinium oxycoccus* IN HCl SOLUTIONS
November 23 to December 17, 1913

Culture solution	Amount of water in grams			Gain or loss in weight of plants (in grams)
	Absorbed	Transpired	Retained	
1. Bog water	3.45	3.10	0.35	0.35
2. *n*/400 HCl	6.15	5.95	0.20	0.20
3. *n*/3,200 HCl	6.20	6.00	0.20	0.20
4. *n*/6,400 HCl	5.10	4.85	0.25	0.25
5. *n*/12,800 HCl	4.85	4.45	0.40	0.40

Plants in bog water with long, thin roots, devoid of roothairs. Root growth retarded in strong acid solutions; in weaker solutions the rootcap consists of loose tissue, as if gelatinized. Atmospheric conditions as in Table VIII.

decrease in weight increment may arise through faulty nutrition and enforce compensating processes.

8. Weaker acid solutions are more effective in increasing the hydration capacity of tissues than stronger concentrations of acids, and thus are more efficient in increasing (stimulating) transpiration but not the growth of plants. The variations cannot always be attributed to differences in the absorptive surface of the root system. But whether the variations in the amount of water absorbed and held by tissues are essentially an expression of their colloidal state, or of ion concentration (osmotic pressure), or connected with hydrolytic reactions and hence with metabolism, needs further inquiry.

9. The amount of water *retained* within the plant and involved in metabolism and in the growth increment should be known as the *physiological* water requirement, to distinguish it from the other term used on the basis of the environmental water relation of the plant.

TABLE VII

TRANSPIRATION AND GROWTH OF *Coleus* sp. IN GLYCOCOLL SOLUTIONS
December 17, 1913, to March 1, 1914

Culture solution	Amount of water in grams			Gain or loss in weight of plants (in grams)
	Absorbed	Transpired	Retained	
1. Distilled water	14.70	13.65	1.15	1.95
	21.50	21.10	0.40	
	25.50	25.20	0.30	
	45.00	46.60	−1.60	−1.60
2. *n*/800 glycocoll	7.20	7.00	0.20	1.13
	10.00	9.80	0.20	
	23.70	23.20	0.50	
	34.20	35.10	−0.90	−0.90
3. *n*/1,600 glycocoll	9.80	9.80	0.00	0.85
	22.60	21.90	0.70	
	27.40	27.40	0.00	
	32.40	33.00	−0.60	−0.60
4. *n*/3,200 glycocoll	30.60	30.10	0.50	1.52
	15.60	15.10	0.50	
	23.60	23.20	0.40	
	23.00	24.70	−1.70	−1.70
5. *n*/6,400 glycocoll	50.80	49.60	1.20	2.72
	25.20	24.50	0.70	
	52.30	53.00	−0.70	−2.00
	30.30	31.50	−1.30	

Immersed portions of stem show strong curvature. Root growth best in solution No. 3. Leaves reduced in size in strong solutions; vary in form in the weaker solutions. Atmospheric conditions up to January 17, as in Table I.

The numerical relation between the quantity of water retained and the amount of growth, *i. e.*, the difference between the initial and final green or dry weight of the plant, may well be termed the coefficient of growth. This value may serve as a criterion for problems in dynamic ecology and in agriculture.

10. The retention of water is the physiological function correlated with and indispensable to growth in general, and to survival and greater areal distribution of the plants entering physically or physiologically arid habitats.

TABLE VIII.

TRANSPIRATION AND GROWTH OF *Coleus* sp. IN HCl SOLUTIONS
November 23 to December 2, 1913

Culture solution	Amount of water in grams			Gain or loss in weight of plants (in grams)
	Absorbed	Transpired	Retained	
1. Bog water	4.18	3.95	0.23	0.23
2. *n*/400 HCl	6.55	6.15	0.40	0.40
3. *n*/800 HCl	29.65	29.10	0.55	0.55
4. *n*/1,600 HCl	14.15	13.50	0.65	0.65
5. *n*/3,200 HCl	5.85	5.55	0.30	0.30
6. *n*/12,800 HCl	4.15	3.85	0.30	0.30

Color of leaves dark green in bog water, lessening in intensity as solutions decrease in concentration. Immersed portion of stem curved in stronger acid solutions and without roots; more or less gelatinized and brown. Roots and roothairs in weaker solutions; with brownish tips. Atmometer, 76.5 cc. Temperature, 8°–28° C. Relative humidity, 40%–98%. Barometer, 29.4–29.9 cm.

TABLE IX

TRANSPIRATION AND GROWTH OF WHEAT SEEDLINGS (*Triticum vulgare*) IN GLYCOCOLL SOLUTIONS

October 28 to November 5, 1913

Culture solution in duplicate series	Transpiration in grams	Green weight of plants	Dry weight of plants
1. Distilled water	81.67	5.030	0.485
2. *n*/800 glycocoll	62.40	4.935	0.580
3. *n*/1,600 glycocoll	60.05	4.480	0.515
4. *n*/3,200 glycocoll	73.10	4.980	0.535
5. *n*/6,400 glycocoll	82.75	5.415	0.515
6. *n*/12,800 glycocoll	85.10	6.655	0.535

Atmometer, 143 cc. Temperature, 15°–37° C. Relative humidity, 40%–83%.

Various other phases of the rôle of retained water—features which must enter into a conception of growth embodying the interplay and correlation of organic parts, their time relation, their changes in form and size, and their metabolism of materials and energy—have been considered briefly in another paper (8). The amount of water retained in cells and tissues determines which way biochemical reactions shall go, and it therefore becomes possible to apply this method directly to reactions which involve normal activtiy, such as respiration, pigmentation, growth curvatures, etc., as well as pathological conditions leading to death.

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THE AUXO-THERMAL INTEGRATION OF CLIMATIC COMPLEXES[1]

D. T. MacDougal

Some features of the life of a plant depend so largely upon a simple and uncomplicated enzymatic action, oxidation or other form of energy release, reduction, absorption or hydratation that their course may run parallel to that of a reaction the velocity of which is known and expressible by exponential law. As examples of this may be cited the development of buds or the germination of seeds in which the hydrolysis of accumulated food-material and the measurable development ensue at a rate in accordance with van't Hof's rule by which the velocity increases two or three times with every rise of temperature of 18° F.[2]

Many of the more important activities of living matter, however, are the combined expression of a complicated group of reactions, in which the initiating temperature, and the acceleration above it are not identical or parallel, and these may be linked with still others which are not a function of temperature.

It follows therefore that the resultant may be one not calculable from known data concerning reaction velocities. This is true of growth, differentiation and of the constructive processes in general, both in a morphogenic and physiologic sense.

It is obvious that if we are to make any rational interpretation of the entire effect of temperature upon the organism in any phase of its activity, or during all of its ontogeny a method must be formulated by which the effect of the duration and intensity of the temperature exposure upon the organism may be calculated. Now since this may not be done in terms of the reaction velocity of any of the underlying or component chemico-physical activities our only recourse is to use a resultant standard, one derived from the organism itself. In other words, it is proposed to measure climate in terms of protoplasmic activity, a procedure that has become necessary in the experimental tests of the effect of climatic complexes upon plants now being carried out at the Desert and Coastal Laboratories and the attached plantations. If any rational analysis is to be made of the direct and inheritable effects that have come under observation in this work it must be done upon the basis of determinations of the influence of the separate environic components, of which of course temperature is one of the most important.

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[1] Paper read in the Symposium on "Temperature Effects" before the Botanical Society of America at Atlanta, December 31, 1913.

[2] See Drinkard, Fruit-bud Formation and Development. Ann. Rep. Va. Polytech. Inst. Agr. Exper. Sta. 206–212, 1909 and 1910, as an example of the application of physio-chemical constants to developmental processes.

The author's work led him to a realization of the necessity for such a method in 1900 and the first attempts at anything definite were presented at the Denver meeting of the A. A. A. S. in 1901. The method then proposed consisted simply in estimating the area of the thermographic diagram by the line of freezing point and by the temperature tracing from the beginning of a season until a plant had attained a certain stage of its development. The vertical component in such figures being degrees of temperature, and the horizontal element being elapsed time, the resulting amounts were designated as *hour-degrees*. Thus the silver maple (*Acer saccharinum*) was found to have been exposed to 3,466 hour-degree units from the winter solstice until the time of blooming on March 26, 1901. *Draba verna* reached a similar state in 974 hours after an exposure of 1,644 units of exposure.[3]

This method was superior to all previous methods of summation of temperature-effects in that it gave full value to the time factor of exposure, which the older methods of cumulation, or of totalizing maxima or averages of daily temperatures did not. It was obviously a purely empirical procedure, as growth at all temperatures above the freezing was taken as uniform. The use of the freezing point as a physiological zero has much in its favor in phenological observations such as those upon Acer and Draba noted above, but a method of wider usefulness would assume a zero above this point.

As applied to the possibilities of growth it gave a value for the year of 78,836 hour-degree units to a meadow in the New York Botanical Garden and to the floor of the hemlock forest within four hundred yards of 68,596 units, a basis of comparison generally in harmony with the results of observations upon temperature and growth in the two places.[4]

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[3] MacDougal, D. T. The temperature of the soil. Jour. N. Y. Bot. Garden **3**: 125. 1902, and Factors affecting the seasonal activities of plants. Plant World **10**: 218. 1907.

Livingston has recently used a modified summation method dealing with temperature effects in phytogeography by which the mean normal temperatures are evaluated in terms of a reaction velocity assumed to be doubled for every rise of 18° F.,[5] and the results correlated with the occurrence of dominant types of vegetation in America. Distribution however, is, so far as temperatures are concerned, a function of growth or development possibilities and of endurance of maxima and minima as has been pointed out by Dr. Shreve. This method is then also to be classed as an empirical procedure since the rate of growth of a plant through its temperature range is not to be expressed in a single formula, and the rate during part of the tonic range is much greater than that assumed. Thus the temperature coefficient of growth of *Lupinus albus* is 3.7 between 14.4 and 24.2° C, 4.9 between 17 and 26° C., while that of *Zea mais* is 5.3 between 24.2 and 29° C., and 6.4 between 23.5 and 33.5° C. The rate of growth of wheat (*Triticum vulgare*) between its minimum of 40° F. and 58° F. is 5, and the temperature coefficient from 58 to 76° F. is 11, as may be seen by reference to figure 1.

The first step in the selection of a standard by which the constructive processes of organisms might be measured consists in fixing upon some form of activity, which is delicately affected by temperature and is readily measurable. Growth-extension or expansion seems to meet these requirements most fully, and this selection has the additional advantage that a large number of measurements of the actual rate in several species are already available.

The graphs representing the rate of growth of the higher plants show that these in general begin with a *miminum* variously placed with respect to several species, that the rate runs through several degrees of temperature with but little increase, then at a certain point the acceleration with the rise in temperature is extremely rapid until an *optimum* is reached. If the temperature rises above this point growth and development decrease and rapidly decline to zero.

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[4] MacDougal, D. T. The seasonal activities of plants; factors affecting distribution and development. Sc. Am. Suppl., October 17, p. 251, 1908.

[5] See Livingston, B. E. Temperature Coefficient in Plant Geography and Climatology, Bot. Gaz. **56**: 349–375, 1913. The titles of several papers relative to reaction velocity in organisms are cited by this author.

These critical points are so widely apart (as much as 25° F. in various species) that anything like a generalized expression of the course or rate of growth of the higher plants would have but little value as a standard for the derivation of a unit for measuring the biologic effects of climate. It was therefore determined that evaluation of temperature exposures must for the present be made in terms of the activity of some single plant, and it may well fall out later that the generalization from a group of species may be of greater value. Many reasons make the data obtained from the growth of monocotyledons more valuable for the purpose mentioned, and several series of measurements of the rate of growth of wheat made by Köppen, deVries and others being available, these were used for testing the method proposed for estimating the temperature factor in the climate of a place. It is to be noted however that these measurements were made on the roots, "hypocotyls" or other parts of young plants, for the most part under equable conditions and it may not be assumed that the illumination or humidity was in all cases under good control. The available data however show that growth elongation begins at about 40° F., rises very slowly in rate to about 63° F., then accelerates rapidly to 86.5° F. with however a break or check between 80° and 83° F. The rate of growth at the highest temperature given was found to be 105 mm. in 48 hours, but, above this, growth is checked and during a further rise of 11° the rate falls to 5.4 and comes to zero at 108° F. (see figure 1). The first check in the rate of growth above 80° F. seems to have been found in several species and the most rational suggestion is that it may be attributed to some change in phase of the colloids, perhaps of the suspensoids. The final comprehensive drop follows a course highly suggestive of the procedure in which invertases and other specific substances are destroyed by high temperatures or their action inhibited.

An extensive series of calibrations have already been begun at the Desert Laboratory and at the Coastal Laboratory in which the rate of growth of the internodes from the upper part of the stem will be measured in such manner as to give the possibility of some interpretation of both the temporary and final retardation of growth in terms of physico-chemical processes. The data already at hand may be used for the present, and if our measurements differ from them the new factors may be applied to the figures obtained from the thermograph sheets in place of those expressed in the graph shown in figure 1.

Assuming now that it is desired to evaluate the variable temperature of any place or of any experimental setting it is first of all necessary to secure a reliable thermograph record for the period under investigation which might include the entire frostless season or the time in which a certain stage of development of selected organisms had been accomplished. Next, this record is ruled by lines which will divide it into figures, the area of which, measured by a planimeter, represents the length of time applied to the intensity of the temperature. The obvious procedure is simply to construct regular figures which shall include the area of the thermographic diagram as nearly as possible and to make these figures of such size that the use of averaged rates of growth will include the smallest practicable error (see figure 2). Crude as this method may appear in this preliminary form it however deals with actual and observable facts and all of its essentials are capable of correction. The first step in such improvement would of course consist in the re-measurements noted above under conditions approximating the daily range with corrections for the age of the material and with control of all of the environic factors. It may be expected that growth of the upper part of the stem from material newly formed in the leaf-blades will be different from that which might take place in organs in direct connection with the relatively great stores of easily hydrolyzable material in the seeds.

In the case of the wheat plant it has been found most convenient in this preliminary essay to calibrate growth vlaues at the average rate between 40 and 65° F., 65° and 70°, 70° and 75°, 75° and 80°, 80° and 85°, 85° and 92° F., etc. The lowest rate, including a range of 25° F., the areas measured on the thermograph sheet, in every case should have a similar ideal range to avoid distortion of results.

The summation of the results of the use of the planimeter on the thermograph sheets gives the total hour-degrees during a week, month or any other period during which the temperature stood within the limits mentioned and it will then be but necessary to apply to this sum the factor expressing the rate of growth to obtain the relative value of the exposures as is illustrated by the tables below constructed from data obtained at the Desert and Coastal Laboratories.

COASTAL LABORATORY, 1912

January				
	40–65° F.	56.4 × 4.5	254.7	
		1.3 × 20.	26.0	280.7
February				
	40–65	70.8 × 4.5 (28 days)	307.8	
		Total for 29 days..........	318.0	318.0
March				
	40–65	76. × 4.5	342.0	
	65–70	3.4 × 20.	68.0	410.0
April				
	40–65	68.7 × 4.5	308.1	
		1. × 20.	20.	328.1
May				
	40–65	97.2 × 4.5	437.4	
	65–70	4. × 20.	80.	517.4
June				
	40–65	111.4 × 4.5	501.3	
	65–70	23.7 × 20.	474.0	
	70–75	5.0 × 45.	225.0	
	75–80	1.7 × 70.	119.0	
	80–85	1. × 78.	78.	1,379.3
		Total....................		3,233.5

COASTAL LABORATORY, 1913

January				
	40–65° F.	47. × 4.5	211.5	211.5
February				
	40–65	60. × 4.5	270.	
	65–70	1.5 × 20.	30.	300.0
March				
	40–65	62.3 × 4.5	280.4	
	65–70	9.7 × 20.	194.0	
	70–75	1.5 × 45.	67.5	541.9
April				
	40–65	79. × 4.5	355.	
	65–70	4.2 × 20.	84.	439.5
May				
	40–65	99.2 × 4.5	446.4	
	65–70	12.3 × 20.	246.0	692.4
June				
	40–65	103.7 × 4.5	466.7	
	65–70	15.6 × 20.	312.0	
	70–75	2.9 × 45.	131.5	
	75–80	1. × 70.	70.	980.2
		Total..................		3,165.5

DESERT LABORATORY, 1912

January				
	40–65° F.	120.4 × 4.5 × .8	433.4	
	65–70	20.3 × 20. × .8	324.5	
	70–75	18.6 × 45. × .8	669.6	
	75–80	5.5 × 70. × .8	318.	
		Total		1,745.5
February[6]				
	40–65° F.	75.3 × 4.5 × .8	38.	309.
	65–70	15.8 × 20. × .8	36.1	288.9
	70–75	31.6 × 45. × .8	162.5	1,300.0
	75–80	2.1 × 70. × .8	16.8	134.4
		Total		2,032.3
March				
	40–65° F.	134.3 × 4.5 × .8	483.5	
	65–70	36.7 × 20. × .8	587.2	
	70–75	24.3 × 45. × .8	874.8	
	75–80	8.4 × 70. × .8	470.4	
		Total		2,415.9
April				
	40–65° F.	97.4 × 4.5 × .8	330.6	
	65–70	37.1 × 20. × .8	593.6	
	70–75	37.0 × 45. × .8	1,332.0	
	75–80	16.6 × 70. × .8	929.6	
	80–85	6.5 × 78. × .8	405.6	
	85–90	2.5 × 95. × .8	190.0	
		Total		3,781.4
		Total		9,974.0

DESERT LABORATORY, 1913

January				
	40–65° F.	87.7 × 4.5 × .8	315.7	
	65–70	21.9 × 20. × .8	350.4	
	70–75	17.1 × 45. × .8	615.6	
	75–80	1.4 × 70. × .8	78.4	
		Total		1,360.1
February				
	40–65° F.	106.9 × 4.5 × .8	384.8	
	65–70	19.6 × 20. × .8	313.6	
	70–75	14.9 × 45. × .8	536.4	
	75–80	5.7 × 70. × .8	319.2	
		Total		1,554.0

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[6] The record of five days was missing and the month was taken at 28 days one-seventh being added to bring the amounts up for comparison with other years and other places.

Note: The areas of the sheets at the Desert Laboratory and the Coastal Laboratory are as 5 to 4 and hence data from the first-named place are to be multiplied by .8 to reduce to equivalent terms.

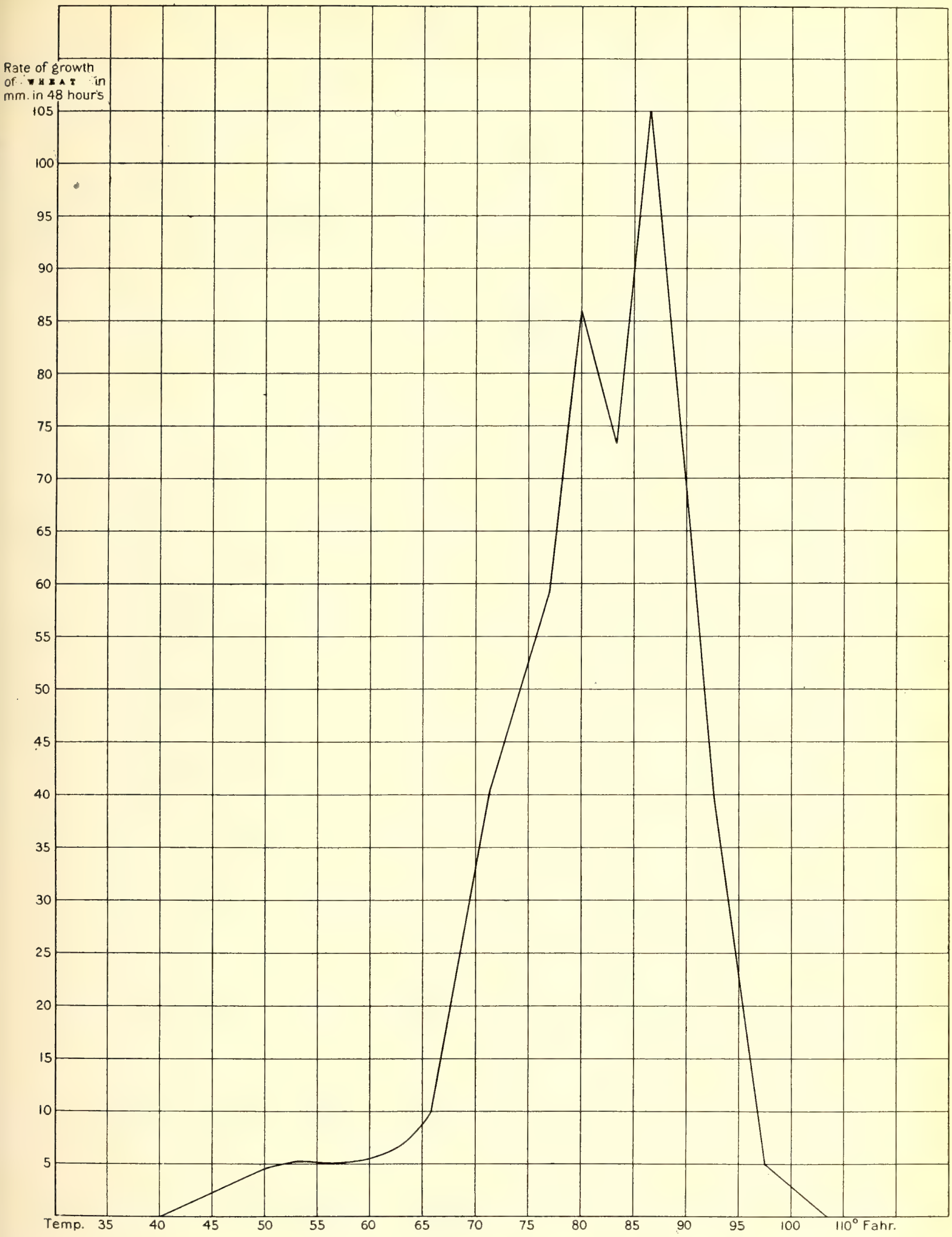

FIG. 1.

MACDOUGAL: CLIMATIC COMPLEXES.

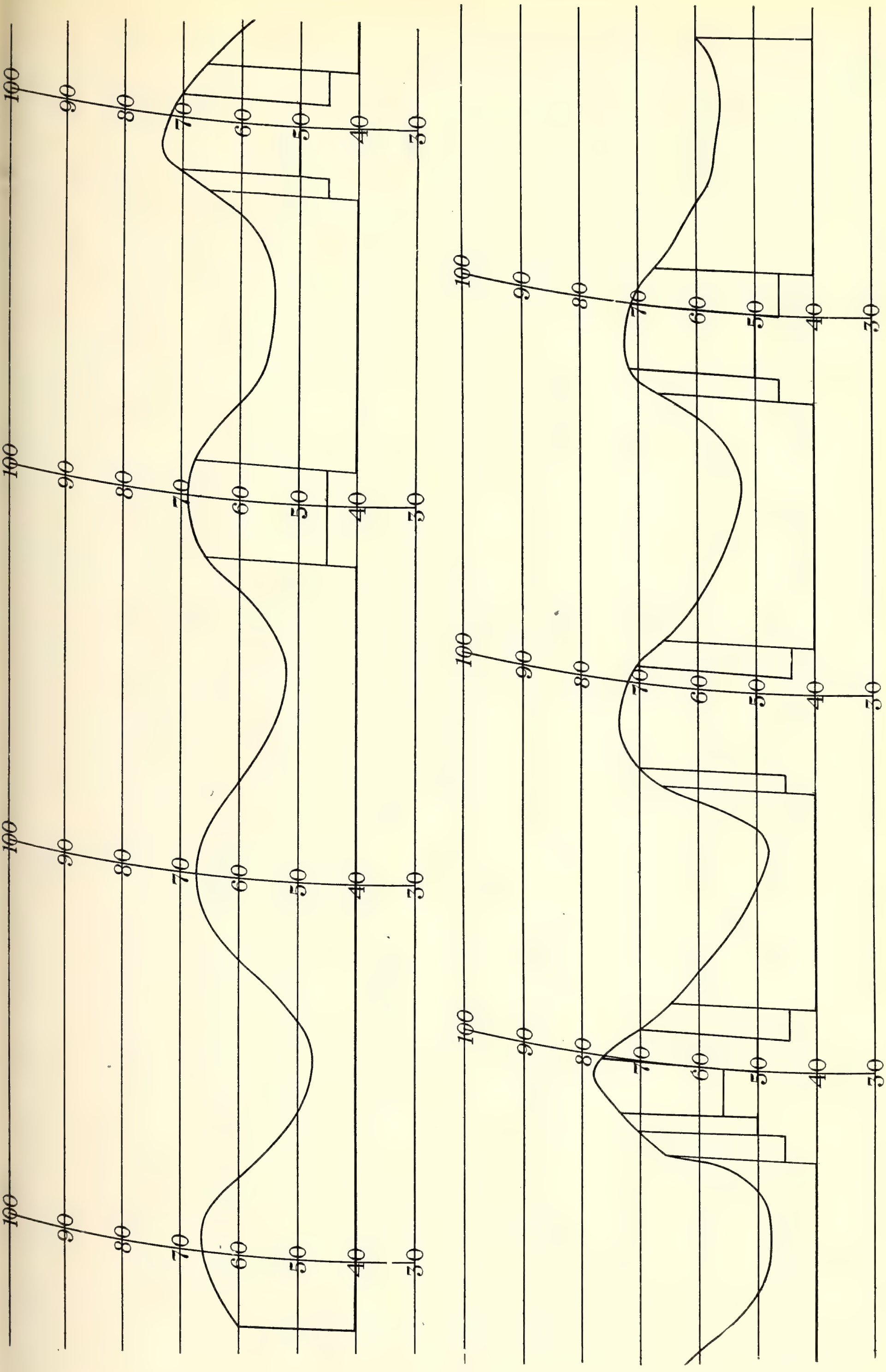

FIG. 2. Thermograph record, Coastal Laboratory, Carmel, California, June 16–23, 1913.

MACDOUGAL: CLIMATIC COMPLEXES.

March			
40–65° F.	80.4 × 4.5 × .8	289.44	
65–70	25.2 × 20. × .8	403.2	
70–75	18.4 × 45. × .8	662.4	
	Add one eighth of above for four days missing record in which the temperature did not rise above 75° F.	169.3	
75–80	4.4 × 70. × .8	246.4	
80–85	1.5 × 78. × .8	93.6	
85–90	1.4 × 85. × .8	96.2	
	Total		1,959.5
April			
40–65° F.	91.9 × 4.5 × .8	330.84	
65–70	37.2 × 20. × .8	595.2	
70–75	37.1 × 45. × .8	1,335.6	
75–80	32.3 × 70. × .8	1,808.8	
80–85	16.6 × 78. × .8	1,035.84	
85–90	2.7 × 95. × .8	167.6	
	Total		5,273.88

The time at my disposal does not allow me to cite illustrations of the manner in which the results of the integration of the temperature given in these tables are correlated with distinctive formative and reproductive reactions in some of the species under critical observation in our experimental grounds. Some of these are notable and striking.

It need only be said in closing that not only may the exposures given above be evaluated in revised terms of growth of the wheat but they may also be converted into terms of growth or activity of any plant which has been the object of the necessary measurements.

Explanation of Plates XVII and XVIII.

Fig. 1. Graph showing relations of growth of wheat to temperature; compiled from data obtained by various authors and cited in Pfeffer's Plant Physiology, Eng. Ed.: 1903.

Fig. 2. Diagram of thermograph record of the Coastal Laboratory, Carmel, California, June 16–23, 1913, divided for measurement of areas to which growth values may be assigned.

THE ROLE OF WINTER TEMPERATURES IN DETERMINING THE DISTRIBUTION OF PLANTS[1]

Forrest Shreve

The investigation of the control of plant distribution by the various phases of the temperature factor is one of the most important tasks of physiological plant geography, at the same time that it is one of the most backward and difficult. The field is an old one, the first outlines of which were sketched by Willdenow, Humboldt and Schouw. They delimited the great temperature zones of the earth, but in relation to flora rather than to vegetation,—in relation to the distribution of species, genera and families, rather than to the great physiologically coherent assemblages of plants. To the investigations and explorations of these men and their immediate successors we owe the dictum (which is itself of later origin) that the character of the flora of a region is controlled by temperature, that of its vegetation by moisture.

The only temperature datum used by the early plant geographers was the annual mean, and this is still used as the sole criterion in distributional studies by men who prefer that their generalizations should be broad rather than of scientific utility. The gigantic toil of the phenologists between 1850 and 1890 yielded some results on the operation of temperature, and gave us a vast accumulation of data of which some real use was made at the time, and to which we may return in future investigations. The men by whom this work was carried on were mostly climatologists, and their efforts were handicapped by the fact that they worked extensively rather than intensively, and that they had not a sufficient foundation of physiological facts upon which to operate.

Our knowledge of temperature influences in the distribution of vegetation is fundamentally underlaid by our knowledge of temperature influences on individual species of plants, and the two bodies of knowledge have come from fields of study which are widely unlike in their perspective and methods: from geography and from plant physiology. The viewpoint of the geographer—and with him that of many floristic plant geographers—is too broad and general to give due regard to the actual physiological effects of temperature on plants; the point of view of the plant physiologist, on the other hand, is often too intensive to enable him to realize that the "conditions" of his laboratory experiment are identical with the "physical factors" of the environment of plants growing under a state of nature, and he is therefore prone to neglect the bearing of his work on the problems of the field. There is no greater desideratum at the present time—with respect to the operation of all environmental factors—than to bring the intensive methods and exactness of logic which characterise physiological work to bear on the large and intricate problems of physiological plant geography.

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[1] Paper read in the Symposium on "Temperature Effects" before the Botanical Society of America at Atlanta, December 31, 1913.

In a consideration of the influence of the various phases of the temperature factor on the limitation of the distribution of plants, and on their relative abundance in different parts of their areas, it will be seen that these phases fall into two well-marked groups. The first to be mentioned—and neither can be first in importance—are those phases of temperature which have to do with the length of the season in which growth and other activities are possible, with the curve of temperature conditions within this season, and with the possible effect of the highest portions of the seasonal curve as deterrent to the activities of plants. The second group of phases of the temperature factor are those which have to do with the length of the season during which low temperatures may exert a deterrent or fatal effect upon physiological activities, and with the duration and intensity of the critical periods in this season.

Considerable physiological study has been given to the effect of gradients of temperature upon plant activities, and also to the effect of various durations and intensities of cold. Various attempts have been made to formulate the results of these physiological investigations in such a way as to give them general applicability in plant geography, and of these attempts it may be said in general that each has been an improvement upon its predecessors. It has been one of the most common errors of the phenologists that they have considered a degree at one part of the temperature scale the equivalent of a degree at another part of the scale. This impossible assumption has led to the totalling of daily mean temperatures as the means of securing an index of the possibilities for plant existence or plant growth in a given locality. Thanks to the growing interest manifested in this subject by men with physiological training, the crudities of the earlier work are being eliminated from the most recent utterances on this subject.

One of the most widely used schemes for the formulation of temperature data in such manner as to give them general applicability in biogeography, is the system of life zones proposed by Merriam for North America. These zones are based on the isothermal lines which indicate the totalled degrees of temperature for the growing season. Not only does the placing of such lines rest fundamentally upon the assumption that every degree of temperature is the equivalent of every other degree in respect of plant activity, but it takes no account whatever of the second phase of temperature effects which are exerted in the frost season.

Not only is Merriam's scheme of life zones to be criticized as a geographical delineation of temperature conditions, but it is even more fundamentally faulty when it is urged as a general scheme of classification of biogeographical regions. In spite of the importance of temperature as a factor in distribution it is illogical to take it as the sole criterion for the limits of distributional regions, especially when the rôle of soil and atmospheric moisture is so obviously of vital importance and is so potent in determining the areas of the principal vegetational regions of the globe.

The temperature phases of the growing season and those of the frost season are in no respect reciprocal or complementary to each other, excepting in the mere matter of the length of the two seasons. The end effects of the temperature conditions of summer and those of winter are quite distinct as they are registered in the limitation of the range of species. In the case of plants which range over large areas it is quite commonly the winter phases of temperature which limit their northward distribution, and the summer phases which restrict them at their southern edge. In many other cases it is the summer phases which determine the northern limit of the conditions that make it possible for a species to grow.

More attention has been given by phenologists to the temperature phases of the growing season, and their potentialities, than to those of the frost season. The writer has welcomed, therefore, an opportunity to make some preliminary investigations of the importance of the temperature phases of the frost season in determining the distributional limits of some sub-tropical desert plants.

The Desert Laboratory is situated near the northern edge of a botanical province which extends far southward into Mexico, and includes in its flora many Mexican plants which do not penetrate more than a few hundred miles into the United States. Within easy access of the Laboratory are three ranges of mountains which rise from the desert floor, at 3,000 ft., to elevations of over 9,000 ft. The slopes of these ranges present rapid gradations of climate and successive changes in vegetation, from cacti and thorn-shrubs at the base, through junipers and evergreen oaks to extended forests of yellow pine, and above them to heavy stands of spruce and fir. The lower limit of the juniper-oak chaparral is about 4,500 ft., that of the pine forest about 6,500 ft. The rugged topography of the slopes gives alternations of exposure and local departures from the normal gradients of both climate and vegetation, which greatly heighten the interest and fruitfulness of the mountains for investigation.

The lower limit of the chaparral and forest plants, and their failure to reach the desert, is to be attributed to the ratio between the soil moisture and the evaporation in the early summer, which is a period of extreme aridity below an elevation of 5,000 ft. It is not logical to dismiss the possibility that some phase of the summer temperature conditions may operate also to limit the distribution of mountain plants at the edge of the desert. Even if this were found to be the case, the results would bear scrutiny in the light of recent work done at the Desert Laboratory which demonstrates a close relation between the temperature of the aerial organs of plants and the maintenance of their absorption-transpiration balance.

The upward limitation of the subtropical desert species is to be attributed to the winter phases of the temperature conditions, as has been determined both by experimental evidence and by correlation of the results of instrumentation with observations of the vertical limits of species.

An attempt to determine the normal temperature gradient in the Santa Catalina Mountains from 3,000 to 8,000 ft. disclosed the very great importance of inversions of temperature in causing local departures from the normal gradient. The rapid nocturnal cooling of the desert soil—which is hastened by its dryness, its prevailing stony or sandy character, and its scant cover of vegetation—is responsible for a pronounced settling of cold air into the valleys and depressions, where it possesses a flow and a definite depth in close analogy to streams of water. A comparison of the average monthly minimum temperatures which prevail at the Desert Laboratory and in the bottom of the Santa Cruz valley, has already been published by the writer.[2] The extensive drainage basin of the Santa Cruz, which is bordered by high ranges of mountains, receives the cold air flow of such a large area that the minimum temperatures in the floor of the valley are always much below those of the Desert Laboratory, situated 335 ft. above the valley, but only half a mile from its edge. The difference between the valley and laboratory minima is greatest on the clear, windless nights of spring and autumn, when the valley temperature has been as much as 31° F. (30° and 61°) below that of the laboratory. The difference between the monthly mean minima for May is 17.8°, that for June 14.4°, both of these months being dry and their nights prevailingly clear. On cloudy nights after heavy rains the minima of the two places have approached within 2° (69° and 71°), that of the valley being lower. The difference between the monthly mean minima for July and August are respectively 7.7° and 8.8°, these months being relatively cloudy and damp.

In Soldier Cañon, at an elevation of 5,000 ft. in the Santa Catalina Mountains, the temperature of the floor of the cañon has been observed to be 8° below that of the slope of the cañon 100 ft. above the floor. In Bear Cañon, at 6,000 ft. elevation in the same range, the minimum in the floor of the cañon has been observed 7° lower than the minimum of the rim of the cañon 1,000 ft. above. Observations 100 ft. above the floor would probably have revealed an even greater difference. The walls of both these cañons are clothed with open stands of desert and chaparral plants, the latter cañon having extremely rocky walls. In Marshall Gulch, at 7,725 ft. elevation in the Santa Catalina Mountains, the minimum temperatures are identical in the bottom of the gulch and on its rim 275 ft. above. The walls of this gulch, or cañon, are heavily covered with forests of pine, spruce and fir. The drainage areas of the three mountain cañons are, in each case, much smaller than that of the Santa Cruz river.

Our knowledge of the conditions which make cold air drainage possible in the desert, leads us to anticipate that it would be less well marked, or even absent, at the heavily forested altitudes of the desert mountain ranges. The diurnal heating of the soil and other surfaces is not so great in the forest, and the nocturnal radiation is retarded not only by the forest cover but by the surface litter of the soil, and by the greater humus content and moisture content of the soil.

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[2] Shreve, Forrest. Cold Air Drainage. The Plant World **15**: 110–115, 1912.

The marked character of the temperature inversions has made it necessary, in determining the temperature gradients of the Santa Catalina Mountains, to compare only the stations which are in topographically similar situations. It also makes it necessary to compare separately the gradients of the desert and those of the forested altitudes.

The absolute minimum temperatures of the winter of 1912–13 at several mountain stations showed that there was a successive fall of minimum from 17° at the Desert Laboratory (2,663 ft.) to 13° at 4,000 ft., and − 6° at 6,000 ft. There was then a rise of minimum to .5° at 7,000 ft., the first station in the forest, and a fall to − 2° at 7,725 ft. (see Fig. 1). Similar figures were secured in the winter of 1911–12,

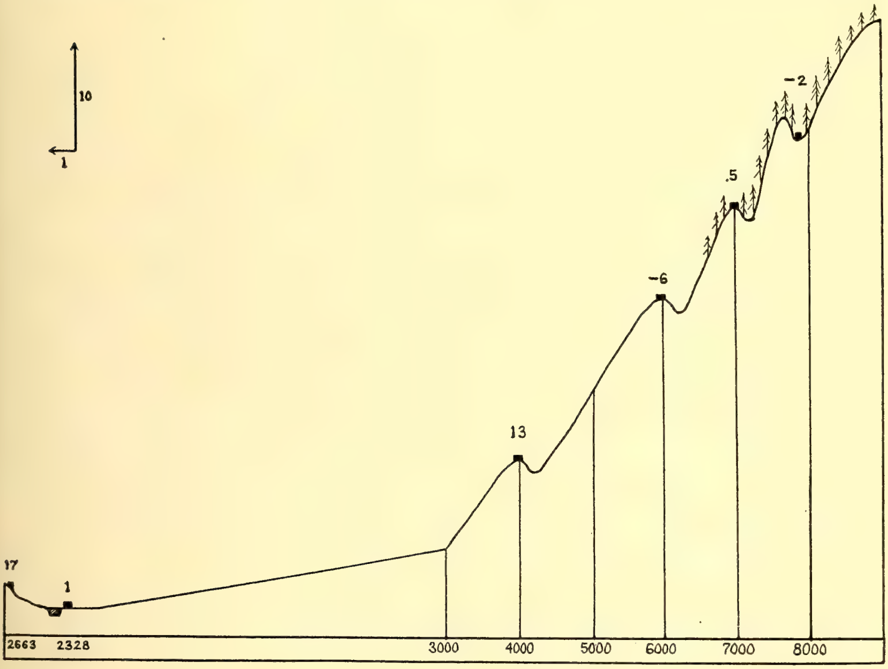

FIG. 1. A vertically exaggerated section from the Desert Laboratory to the summit of the Santa Catalina mountains, to show the elevation and topographic location of temperature stations, together with the absolute minima of the winter of 1912–13.

showing the lowest absolute minimum for the mountain to occur not at the highest station, but at the highest station below the forested elevations. The absolute minimum in the heavy timber at Marshall Gulch was 4° higher than it was 1,700 ft. below in an open stand of manzanitas and oaks, a phenomenon in which cold air drainage was not concerned, since the higher station was in the timber and the lower on a ridge.

The strongly defined character of the cold air drainage of the Santa Cruz valley can be appreciated from the fact that the absolute minimum recorded in the valley was 1°, only three degrees higher than the absolute minimum 5,400 ft. above in the forested region of the Santa Catalina Mountains.

The mean gradient of fall in temperature with increase of altitude, averaged for a large number of mountains in different latitudes and climates, indicates a fall of 3.46° per 1,000 ft. This gradient happens to be identical with that for Pikes Peak. The steepest gradient previously recorded is 4.12° per 1,000 ft., for the western slope of the Sierra Nevada. The gradient for the Santa Catalina Mountains has been determined by comparing the daily mean temperatures of the Desert Laboratory with the daily means secured from observations at the Montane Plantation of the Laboratory and at Mt. Lemmon (alt. 9,150 ft.). This comparison evades the influence of cold air drainage by reason of the fact that both the laboratory and the forested altitudes are beyond its effects. The value of the gradient thus determined is 5.2°, or more than one degree higher than the highest gradient previously reported, and differing from the maximum reported gradient more than the said maximum differs from the mean of all reported gradients.

The gradient derived from the absolute winter minima of the Desert Laboratory and of the ridge stations at 4,000 and 6,000 ft. is 6.6° per 1,000 ft. The gradient above the commencement of timber is 2.5° per 1,000 ft. The gradient of temperature fall in the free air has been determined at the Blue Hill Observatory, for low altitudes, as 2.16° per 1,000 ft.

The streams of cold air which flow from the mountain cañons are shallow, never exceeding 75 ft. in depth, and often being less than 50 ft. Below elevations of 6,000 ft., by virtue of these streams, the minimum temperature conditions of cañons and other topographic depressions are equivalent to those of ridges and slopes which lie about 2,000 ft. higher. The vertical limits of a large number of species have been found to differ by this amount as determined for cañons and for ridges and slopes.

It would appear, therefore, that the winter phases of the temperature factor sustain, in their distribution, a close relation to the distribution of plants. The experimental work carried out to test this correlation was planned only after a study of the relation existing between the vertical distribution of several plants throughout Arizona, and the vertical distribution of several phases of low temperature. Data were secured from a series of Weather Bureau stations extending from the low southwestern corner of Arizona to the cool highlands of the northern part of the state. The length of the frost season, the number of days with freezing temperature, the total number of hours (per winter) of below-freezing temperature, the greatest number of consecutive hours of frost, and the lowest minimum have all been ascertained, not in their average, but in their maximum intensity. The results indicated that the greatest number of consecutive hours of freezing temperature is the factor most closely corresponding, in its distribution, with the limitation of the species concerned.

Experiments performed[3] with succulent plants native to various altitudes in southern Arizona, indicated that the number of hours that they are exposed to temperatures below freezing determines their death, without regard to the absolute minimum reached during the freezing period (although minima below 18° F. were not used). The succulents which have the lowest vertical limit are unable to resist freezing over 19 to 22 hours in duration, while the species of higher and higher limits are progressively able to withstand longer and longer periods of freezing, up to 66 hours. With the limited hardiness of the Arizona species of cacti may be compared the behavior of *Opuntia missouriensis*, which withstood 375 consecutive hours of freezing temperature in the winter of 1910–11, at Havre, Montana, near the northernmost limit of the succulent type of plant.

There are doubtless species other than those investigated in which the absolute minimum attained during freezing is of critical importance in causing death; there are doubtless very low minima (never attained in Arizona) which would be fatal on very short exposure; and there are probably still other species of plants for which some of the other winter temperature phases mentioned are of paramount importance as limiting factors.

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[3] Shreve, Forrest. The Influence of Low Temperatures on the Distribution of the Giant Cactus. The Plant World **14**: 136–146, 1911.

It is impossible to speak authoritatively of the importance of the temperature conditions of the growing season or those of the frost season in their relation to vegetation as a whole until we posess a large body of data regarding their influence on individual species. It is fundamentally important, furthermore, that our knowledge of the influence of these and other physical factors should be secured by observation of the distributional limits and behavior of species, by instrumentation, calculated to single out the critical factors and their critical intensities, and finally by investigation, in the laboratory, of the operation of these factors and of these intensities. Only in some such manner as this is it possible actually to interpret the underlying causes of the phenomena of distribution. Thus only can the observed or the instrumentally determined correlations of the field be given confirmation. Thus only can physiological plant geography place its generalizations on a secure logical basis.

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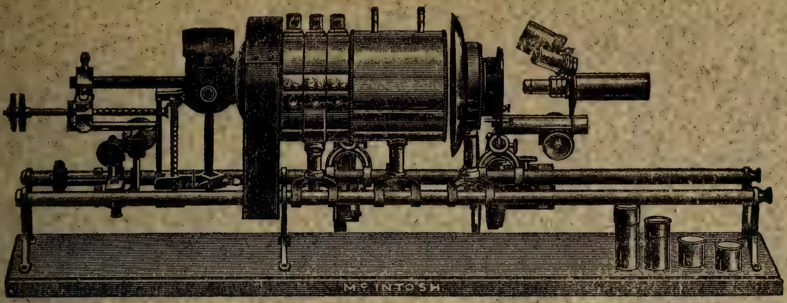

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THE INFLUENCE OF TEMPERATURE ON CHEMICAL REACTION IN GENERAL*

FREDERICK BARRY

Any inquiry into the effect of a change of temperature upon a physical or chemical process involves at the outset considerations of a fundamental character as to the nature of physical change in general. In order that the point of view from which it is habitual for the physicist or the chemist to examine such a question may be clearly defined, so that the short discussion which follows may not be too much obscured by its necessary brevity, I shall venture at once to summarize that group of general conceptions in terms of which physical processes are most understandingly described and correlated. Familiar though these ideas may be to us all, we shall thus recall more vividly than we otherwise might that habit of thought which alone has been found serviceable to the practical understanding of natural phenomena, and the character of its premises.

As a result of the long-continued series of investigations which we associate with the immortal names of Rumford, Mayer, Helmholtz, and Joule, we have come to look upon all the natural occurrences that fall within the range of our experience as instances of a continuous change in the distribution of that which we call energy; of which mechanical work, heat, electrification are diverse but interconvertible manifestations. It would be gratuitous in this place to trace the steps by which men slowly came to a full realization of the significance of this remarkable concept, by which phenomena so dissimilar could be grouped together as of one essential quality. Nor is it necessary to dwell upon the tremendous value of such an idea—derived wholly

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* Paper read in the Symposium on "Temperature Effects" before the Botanical Society of America at Atlanta, Dec. 31, 1913.

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from experience, and in none of its implications at variance with the results of experiment—which enables man to correlate the great complexity of all measurable phenomena as interdependent changes, of one general and definite character within a single vast mechanism.

We know, concerning energy, that within the limits set by the possible accuracy of our measurements, it suffers no diminution with the passage of time; that its disappearance in one form is coincident with its appearance in another. We are able to formulate exactly its dimensions: that is, its magnitude in terms of the fundamental concepts of mass, and of geometrical space and time. We know, further—thanks to the invaluable labors of Carnot and Clausius—that its possible transformation is limited in a significant way: that while all of the energy forms familiar to us may be converted completely into heat, heat energy itself can be only partially transformed. It results that within any isolated system, as time goes on, though no energy disappears, an increasing portion of it becomes unavialable, in the form of uniformly distributed heat. We are thus compelled in studying the energy changes which accompany any natural process to distinguish between that part of the heat energy released or absorbed which may be transformed directly or indirectly into work, and that which may not; to introduce into our scheme two additional conceptions, those of free energy and bound energy.

Our knowledge of energy thus summarized is expressed in two laws, which are fundamental: the law of the conservation of energy, and the law of the limited transformation of energy. These are commonly known, respectively, as the first and second laws of thermodynamics. Both have been quantitatively formulated in terms of free and bound energies; and these formulations will soon claim our attention (1).

Both thermodynamical laws, because of their very generality, are characterized by one limitation: they apply to phenomenal changes in the gross, and are insufficient to describe in any satisfactory way, the details of a natural process. The need thus indicated has, however, been almost completely satisfied by the elaborate conceptual scheme which during the last century has been developed—simultaneously with the theory of energetics, and consistently with the growth of chemical knowledge—out of Dalton's first scientific application of the atomistic conception of matter. In the modern kinetic-molecular theory, already worked out in great perfection of detail, the physical scientist has a means of supplementing and extending the implications of his laws of energetics, and of intelligibly presenting interpretations consistent with them, of phenomena which actually lie beyond the range of their possible application (2).

This is not the time or place to discuss the question, whether or not it is justifiable to interpret all natural processes mechanically. It is pertinent only to remark that each step taken in the development of the mechanistic view has been necessitated by man's inability to frame other workable interpretations of the facts; so that in our own time it results that the only apparently tenable criticism of this view of nature is one which bases its opposition on the limitations of the intellect itself.[1]

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[1] The philosophical speculations on this point, and the lively discussions incident to them which have occupied so much recent attention are of the utmost interest to any student of current thought; and it is by no means intended that such summary reference to them be construed as dismissing them with a gesture. Every discussion of this sort cannot fail to be of the utmost service to the physical scientist, who, in consequence of the very perfection of his conceptual scheme, is too often led to ignore the possible limitations of his method and the metaphysical nature of his habitual assumptions; and is sometimes betrayed into didactic utterance by his uneasy impatience when these are subjected to analysis. The point which it seems is to be kept clearly in mind, however, is this: that it is not necessary for the physicist in defense of his method to consider the strictly philosophical import of his results. Whether his mechanistic notions imply the subsistence of a corresponding Reality or whether they do not, is for such purpose immaterial. The structure of co-ordinated knowledge which has been built on the basis of these concepts has proved itself to be the most powerful instrument which has ever served man's purpose to control his environment. In short, it works; as inevitably it must since every stage in its construction has been built with reference to that scientific criterion which in wider application is now often referred to as the pragmatic rule; and its justification need be sought in nothing more remote than this complete practicality. If there shall be developed in connection with any other habit of thought, such as the vitalistic interpretation of such phenomena as we are here considering, a workable method, by means of which one may be permitted to do something more than meditate upon such matters, then science will be thereby tremendously enriched; and any equable person will be more stimulated than depressed by the ensuing conflict of opinion. It must be admitted that the history of such conceptions, from the demonology of the ancients to that vitalism of our forefathers which was so fortunately anaesthetized early in the last century, holds out little hope of such result. Meanwhile, nevertheless, the service rendered the physicist by well-considered criticism of his tenets from whatever point of view, will be invaluable, in so far as it leads him to a more lively apprehension of the possible limitations of his method, while he holds to his perfectly legitimate purpose of extending his mechanistic interpretations to the point of their furthest possible usefulness.

It is a simple matter to define temperature in terms of these conceptions. According to the kinetic theory of gases, which is the most thoroughly developed application of the atomistic conception of matter—and which in its fundamental assumptions is supported not only by its consistence with fact, but recently by the actual observation and measurement of ceaseless and independent movement among particles approximately of molecular magnitude in colloidal systems—the temperature of a gas is directly proportional to the square of the mean rectilinear velocity of its molecules. More generally, it is a necessary consequence of the fundamental assumptions of this theory that thermal equilibrium between two or more gases indicates the condition that the mean molecular kinetic energies of both or all is the same, and that this is measurable in each system by the product of the molecular mass and the square of its velocity. Since now it is possible for a substance to pass without any abrupt change of condition from the gaseous to the isotropic liquid and amorphous solid state, it is inevitable that we believe this correlation between temperature and mean molecular energy of translation to exist in every state aggregation, assuming only that in crystalline liquids and solids the motion is restricted to a certain definite periodicity (3).

Now, since a change in the total thermal energy of a system is a function not only of the rise in temperature, but also of some specific character of the substance heated—since, in other words, equal increments of total energy do not occasion the same temperature rise in equal masses of different substances—it follows from what has been said that this specific character, the "mass factor" of heat energy, or heat capacity, is a value dependent on the particular constitution of the molecule itself. Consequently, specific heats, which measure the relative heat capacities of equal masses of different substances, become of great theoretical interest.

All are familiar with the success which has attended the attempt to explain chemical relationships and to account for the existence of isomeric substances, especially among the compounds of carbon, by assigning to each substance a definite, though conventional, molecular configuration; and it is equally well known how, in order to explain optical activity and optical isomerism, this representation has taken on a less conventional character, by assuming the existence of geometrical arrangement among the atoms in space (4). No one believes that such formulae represent actual static conditions: too many phenomena point, on the contrary, to the existence of a very lively atomic motion within the molecule; indeed to motion between the parts of the composite atoms (5). There can be no doubt, nevertheless, that the chemist's formulae point in no uncertain way to the actual existence of regular periodic motion of some sort, within the molecule. The specific heats of substances, therefore, represent the varying manner in which an increase in the total energy of a chemical system is distributed between this regular motion within the molecules, and the irregular movements of the molecules themselves.[2]

The most fundamental characteristic of a chemical change, other than the quantitative relationships which obtain between the relative weights of factors and products, is the change in the distribution of energy which accompanies the transformation, and which, indeed, may be said to cause it. In by far the greater number of cases, this redistribution is attended either by the release or by the absorption of measurable quantities of heat. Reactions are thus classified as exothermic and endothermic; and there is good reason for believing that all are so, though perhaps not in measurable degree, excepting only transitions of optical isomers into one another.

The effect of change in temperature on a chemical reaction is, in general, determined: first, by the exothermal or endothermal character; second, by the quantities of heat that are released or absorbed in the transformation of equivalent weights of substance. A study of theinfluence, therefore, must begin with an analysis of these relations.

In the light of the molecular and atomic theories, we must look upon this release or absorption of heat in chemical transformations—this heat of reaction—as measuring the change in the internal energy of the system as it passes from one substance form into another; or, more specifically, as equivalent to the difference between the energies of atomic motion or stress within the molecules of the reacting substances before the change and after. Heats of reaction, therefore, which may be measured calorimetrically in a variety of ways and recently with a high degree of precision, are data of the most fundamental significance. In interpreting these quantities to be the equivalents of intra-molecular energy changes, we have already assumed the operation of the law of the conservation of energy. Another application of this generalization permits us to assert that the internal energy of a molecular system is determined solely by its apparent character at any given moment: that no matter by what path we proceed in forming one compound from another, the total energy change accompanying the transition will be the same. This deduction, known as the law of Hess,[3] permits us to calculate from known data the heat changes which would accompany the formation from their elements of a large number of compounds, even in cases such that the heat of direct combination cannot, for one reason or another, be measured. We have at our command, therefore, a growing accumulation of data, which may be made highly accurate by the general application of recent experimental methods and which define the relative internal energies of a great variety of substances.

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[2] This statement is, of course, too simple. A measured specific heat may possibly include latent heats of transition; but in such cases, the figures do not represent, of course, the specific heats of definite substances, and need to be analyzed further to yield these values. The energy of molecular rotation and of molecular cohesion within the limits defined by any one state of aggregation is in general also included in the values of the specific heats; but this may be legitimately considered to be a function of the molecular constitution and configuration.

From the results of measurements made at different temperatures, these data may now be extended almost at will, to supply a knowledge of the alteration of these values with change of temperature. The same result may be attained, however, by a further application of the first law of energy. This procedure merits attention especially because of the light it throws on the nature of the phenomena. Unfortunately the course of reasoning—which is based on the device of completing an imaginary reversible cyclic process between two temperatures—cannot well be followed out here and now. We shall content outselves with its result—Kirchoff's law—which, though perfectly general, had in this conection best be stated as applied to a chemical change: "The temperature coefficient of a heat of reaction is equal to the difference between the heat capacity of the reacting system before and after the change; is equal, in other words, to the difference between the heat capacities of the factors and the products of the reaction." This difference will, of course, be proportional to the mean specific heats of the substances in question.[4]

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[3] This principle was enunciated by G. H. Hess in 1840, in somewhat simpler terms: to the effect that the heat evolved or absorbed in the formation of any chemical compound from its elements is always the same, whether the synthesis be accomplished directly or by successive reactions. The original statement was a generalization based directly on experiment. Together with the law of Laplace and Lavoisier —formulated fifty years before—which states that the heat evolved in the formation of a compound is equal to that absorbed in its decomposition, it stands as one of the fundamental bases of chemical energetics. Neither generalization, of course, was deduced from the general principle, which in 1840 was not completely formulated. It cannot be doubted nevertheless that in the discovery of these regularities the principle was foreshadowed.

According to the interpretation of specific heat already suggested, this result, independently developed, might have been anticipated from molecular-kinetic considerations. The generalization thus possesses a certain interest, as an instance of the manner in which these two points of view—the thermodynamical and the atomistic—give each other mutual support. Its practical value lies in this: that by its means we may anticipate the probable magnitude of the temperature influence on heat of reaction, by knowledge of the easily measured specific heats of the reacting substances, and of those which are formed in the process.

This influence of temperature is usually not great; though in some single instances it is sufficient to cause reactions which are exothermal at low temperatures to become markedly endothermal at higher Such effects are of course observed only in those cases in which the specific heats of factors and of products are very different, and then only over comparatively wide temperature ranges. In other cases, such as the combustions of organic liquids, though the several specific heats are widely variable, the average specific heats of factors and products are not very different; so that the heat evolved, even over wide ranges of temperature, remains fairly constant. In such cases, moreover, the temperature coefficients of different reactions are similar, so that from the data supplied by measurements of these energy changes the comparative heats of formation of many organic compounds may be deduced with considerable accuracy.

The variation between the specific heats of the substances involved

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[4] The statement is usually formulated (10):

$$\overline{K}_0 - \overline{K} = \frac{U_{T+t} - U_T}{t} \qquad \text{(I)}$$

Where $\overline{K}_0$ is the initial, and $\overline{K}$ the final heat capacity of the system; where t is the temperature change; and where $U_{T+t} - U_T$ represents the change in the total energy within this interval. For small temperature differences the formula may be written

$$\frac{dU}{dT} = \overline{K}_0 - \overline{K}$$

in those reactions which possess the greater present interest are, on the other hand, often very small; so that the corresponding temperature coefficients are comparatively slight. Such is the character, for instance, of reactions between electrolytes in aqueous solution. Finally, in many important types of reactions, the heat evolved is itself so small that until very recently it has certainly not been measurable, although in some instances calculable. Such are dilutions, saponifications and hydrolyses, fermentations—reactions of the greatest interest to the physiologist. Considering, then, that the temperature coefficients of nearly all reactions which take place within the living organism are small, and considering also the relatively narrow temperature ranges within which life can exist, it is readily seen that this effect of temperature, in changing the internal energies of the substance which compose the cell, is very small indeed. The influence, nevertheless, is by no means negligible. It will be shown in the sequel that the shifting of all chemical equilibria is directly dependent upon the change in internal energy that accompanies reaction. While therefore the primary effect of this change, namely the release or absorption of small quantities of heat, may have a relatively slight influence in determining the character of the reactions which take place, it may at the same time cause marked changes in the relative speeds of these reactions. Such effects will undoubtedly have an influence upon the character of such metastable systems. Moreover, in view of the extreme complexity of physiological reactions, particularly with respect to their dependence upon surface adsorption, and enzymotic catalysis, and in consideration of the degree to which such phenomena are influenced by slight changes in temperature, especially in the presence of dissolved electrolytes, it would be a reckless statement to make off hand that the primary influence is itself in any sense negligible.

It cannot now be doubted that the law of the conservation of energy, from which all the general statements here made may be derived, applies to vital as to other processes. The significant and convincing work of Atwater, who in a series of exhaustive calorimetric tests measured the total energy changes in the human body at work and at rest over long periods of time, demonstrated this conclusively.[5]

[5] In Atwater's experiments, the heat evolved by the human body at rest and at work was measured—the subject remaining within a sensitively regulated calorimeter for several hours in each experiment—while at the same time, the weights of the

It becomes, therefore, a matter of more than casual interest to the physiologist that by application of the principle of adiabatic calorimetry, recently developed by T. W. Richards into a method of remarkable accuracy, whereby quantities of heat heretofore unmeasurable may be readily determined, it is possible to extend our knowledge of internal energy changes to regions yet unexplored. Already, specific heats, heats of solution, of neutralization, of dilution, have been measured with a precision impossible a decade ago. In like manner it has been shown that heats of combustion may be determined with sufficient accuracy to show measurable differences between the heats of formation of isomeric substances, and thus to indicate for the first time something of the character of the correspondence between internal energy and molecular configuration.[6] It is not impossible that by extension of this method the heat of slow-moving reactions like saponifications and hydrolyses (if in fact these yield quantities of heat comparable with those set free in the dilution of salt solutions) may be experimentally determined. The results of such work, if positive, would indicate one way among many in which purely physical research may be of service to the biologist.

The foregoing statements summarize in a general way the influence of a change of temperature upon heats of reaction; and thus show the

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products of combustion were determined with the greatest care. The results of twelve rest experiments showed in the average a discrepancy between the heat energy released and that calculated from the heats of combustion of the materials used up of only 0.04 per cent.; in twenty work experiments the average discrepancy was 0.13 per cent.; while that of the whole thirty-two measurements was 0.08 per cent. This degree of precision is comparable with that of the most accurate calorimetric determinations yet made (6).

[6] For instance, heats of neutralization have recently been measured by the adiabatic method with an experimental accuracy of two or three hundredths of a per cent., and the temperature coefficient of a reaction of this type with the remarkably small probable error of one part in ten thousand. In the latter case, the heat of reaction at different temperatures calculated from the experimentally determined coefficient was checked by subsequent observation with this average precision, and with agreement in half of the recorded measurements of six parts in one hundred thousand. The specific heats of liquids, also, have been measured with an accuracy comparable with that reached in the determination of heats of neutralization. Similarly, the heats of combustion of the four isomeric hydrocarbons pseudocumene, mesitylene, normal and isopropyl benzene have been determined to be respectively 1243.5, 1246.4, 1249.0, and 1250.0 kilogram calories, and subject to a probable error no greater than a tenth of the larger differences (7).

character of its effect upon the distribution of total energy in an isolated chemical system. It is now necessary to consider its influence upon speed of reaction and on chemical equilibrium; that is, upon relative intensities of chemical activity, in contrast with the relative differences in internal energies that characterize initial and final states.

The former problem involved a comparison of energy changes taken as wholes—total energy changes; and for its analytical formulation the law of the conservation of energy sufficed. The present inquiry necessitates at the outset an application of the distinction already referred to, between that part of the total energy change which can appear only as gain or loss of heat, and that which may so appear, and yet which may under favorable conditions be converted completely into work. For the tendency of one or more substances by combination or decomposition to form others is clearly not a function of the total energy difference between the two, but rather of that part of it which is capable of transformation not only into heat, but into the work of building up new systems out of old; of transformation not only into irregular molecular motions, but into new types of periodic motion or of stress between the interacting atoms.

Successfully to investigate these phenomena, therefore, we must know how the free energy of a chemical system changes with rise or fall of temperature. This knowledge the second law of thermodynamics supplies. The quantitative formulation of this law, derived from an analysis of reversible processes acting through complete cycles, is (10):

$$\frac{dA}{dT} = \frac{Q}{T} \tag{II}$$

This statement, which has been confirmed by development from different conceptual premises, may be read: "The temperature coefficient of the free energy developed in any isolated natural process $(dA)/(dT)$ is equal to the heat absorbed in that process (Q) divided by the absolute temperature (T). The statement is rendered more intelligible if we combine it with a similar analytical formulation of the law of the conservation of energy in terms of the same quantities. If the corresponding diminution in the total energy in the system be represented by U, this law requires that (10):

$$U = A - Q; \tag{III}$$

That is, "The diminution in the total energy which accompanies any change in an isolated system is exactly equal to the work done by the system, less the heat absorbed."[7]

This statement simply formulates the distinction between free and bound energy. The two expressions may legitimately be combined, since their premises are identical, and their terms have the same meaning in both cases. Thus we may write (10):

$$A - U = T\frac{dA}{dT} \tag{IV}$$

In this expression, we have a quantitative representation of the relation that exists between the free and total energies of a process, in terms of a quantity involving the temperature coefficient of the free energy. Specifically, their difference is equal to this coefficient multiplied by the absolute temperature. Thus, the influence of a change of temperature on the free energy of a process is defined by this formulation, as its influence on the total energy is defined by the law of Kirchoff, though, of course, in dissimilar terms.

Theoretically, then, we have a sufficient analysis of the matter. How now may these generalizations be applied to show the actual influence of temperature change on reaction velocities and on equilibria?

As is well known, it has been experimentally established that the speed of a reaction, if this be conducted isothermally, is proportional to the product of the molecular concentrations of the several substances involved in the reaction. This generalization, the familiar concentration law, needs only to be formulated to be recalled. To illustrate by a simple case: if v equals the speed of a reaction in which equal molecular quantities combine, so conducted that the products are removed as soon as formed, we have:

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[7] Or, "plus the heat evolved." This sounds more natural; for commonly, spontaneous chemical reactions evolve heat and do work by decrease in the total energy of the reacting substances. Of course, in this formulation, U, A, and Q may be either positive or negative quantities; and as seen above it is wholly a matter of taste whether in the first instance A mean work done by the system or upon it; Q, heat evolved or heat absorbed, and so on, providing that the equation be correspondingly written. In the formulation above, U in the positive sense is taken to be a diminution in total energy; because spontaneous reactions at ordinary temperatures are usually attended by such diminution, developing free energy. If, instead of a chemical change, a fusion had been in mind, it would have been more natural to think of an increase in total energy. If, then, U in the positive sense had this meaning, we should have written: $Q = A + U$, or $U = Q - A$, Q and A having the meanings here assigned. Every such formulation becomes clear in a physical sense, only by successive applications to particular cases.

$$v = kc_1c_2\cdots$$

where c_1, c_2, $\cdots$ represent the concentrations of the reacting substances, and where k, the velocity constant, is a constant characteristic of the system in question, which, in comparison with other similar constants, measures relative tendencies to combination. If in a reaction the products accumulate, and if none leave the reacting system, then as soon as one reaction begins with high velocity, the opposing tendency to recombination at once asserts itself. As the one velocity decreases in consequence of diminishing concentration of the factors, the other increases. At any stage of the process the speed of the reaction will be equal to the difference between the two opposed velocities; and when the two become equal, dynamic equilibrium will result. Thus, if the two velocities be written $v_1 = k_1c_Ac_B\cdots$, and, $v_2 = k_2c_{-A'}c_{B'}\cdots$, we have for this condition:

$$v_1 = v_2$$

Whence:

$$\frac{c_{A'}c_{B'}\cdots}{c_Ac_B\cdots} = \frac{k_1}{k_2} = \overline{K} \tag{V}$$

In this formulation (10), $\overline{K}$, known as the equilibrium constant, is the ratio of the two velocity constants, and is characteristic for each isothermal action. It is not necessary to work out the general case, which follows by direct application of the principles involved in the simpler. Since, moreover, irreversible reactions may be legitimately considered to be equilibria very far displaced, we are justified in considering the general expression applicable to all reactions.

The concentration law, first formulated by Guldberg and Waage in 1867, and experimentally verified both by their own investigations and those of previous workers, marked a new epoch in the history of chemistry; for it justified completely the method upheld by Berthollet at the beginning of the century: namely, the application of mechanical concepts to the study of the age long mystery of chemical affinity. The law is of the widest applicability. Its perfect generality is demonstrated by its deduction from the basic assumptions of the molecular theory, as well as by its theoretical derivation from the laws of energy as applied to molecular systems: the common achievement of Horstmann, Willard Gibbs and van t'Hoff. It possesses an independent interest in yielding yet another concordance between the implications of the molecular-kinetic and thermodynamical views of nature; while demonstrating anew the consistence of both with experimental results.

Now, the general effect of temperature changes on chemical equilibrium will be completely expressed by a formulation in thermodynamical terms which shows how the equilibrium constant is thus affected. Such an expression was worked out by van t'Hoff, who, by combining the fundamental equation $A - U = T(dA/dT)$ with that which in the thermodynamical derivation of the Guldberg-Waage equation represents the maximal work of the chemical process, arrived at the expression (10):

$$\frac{dln\overline{K}}{dT} = -\frac{U}{RT^2} \qquad \text{(VI)}$$

This may be read: The negative temperature coefficient of the natural logarithm of the equilibrium constant for any reaction, $dln\overline{K}/dT$, is equal to the total energy change in the process (U) divided by the square of the absolute temperature, times a constant (R).[8]

While the equation of Guldberg and Waage defines the influence of concentration on chemical equilibrium at constant temperature, that of van t'Hoff shows the influence of temperature changes on the equilibrium thus conditioned at constant volume. The tacitly assumed premises of these formulations are easily kept in mind if, following the suggestion of Nernst, we call them respectively the equations of the reaction isotherm and of the reaction isochore. The equation of the isochore in its integrated form[9] has been used in many cases, and successfully, to calculate from known values of the equilibrium constant of a reaction, at different temperatures, the heat of reaction or total energy change. If, conversely, it be used to show the influence of temperature change in shifting chemical equilibrium, most illuminating implications of a general character are brought to notice.

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[8] This is the "gas constant"; which in the equation $pv = (p_0v_0/273)T$ (which describes the behavior of a perfect gas with change of pressure and temperature) equals $p_0v_0/273$. In this formulation, p and v are pressure and volume at T degrees absolute temperature; p_0 is one atmosphere, and v_0 the gram-molecular volume. For other generalizations involving p and v, these quantities may be expressed of course in other units, with the obvious restriction that they shall be comparable magnitudes.

[9] The integral of $dln\overline{K} = -dT(U/RT^2)$ is $ln\overline{K} = (U/RT) + C$, where C is the integration constant. Hence, if $\overline{K}_1$ and $\overline{K}_2$ be values of the equilibrium constant at T_1 and T_2, we get: $ln\overline{K}_2 - ln\overline{K}_1 = U/R(1/T_2 - 1/T_1)$. This procedure presupposes that U over the interval T_1 to T_2 is constant. The approximation involves no appreciable error if this difference is small. If it is large, a general integration must be used.

For instance, we see from the formula that if U, the heat of reaction, is greater than zero, then with increase in temperature, $\bar{K}$ will become smaller; while if U is less than zero, it will become greater. Inasmuch as $\bar{K}$ is always made to indicate by its magnitude the tendency of a reaction in the direction indicated also by the exothermal chemical equation, this means that a rise of temperature will displace a chemical equilibrium in that direction in which the reaction takes place with an absorption of heat.[10] Thus exothermal tendencies will be weakened, and endothermal tendencies strengthened by rise of temperature. This principle, which van t'Hoff called the Law of Mobile Equilibrium, accounts at once for the comparatively greater stability at low temperature of compounds formed by exothermal reaction, and at high temperature of those formed endothermally. By explaining the prevalence of exothermal reactions at ordinary temperatures (which are low even in the experimental temperature range) it gives real significance to the erroneous and much criticized "Principle of Maximal Work" advanced by Thomsen and defended by Berthellot, to the effect that the only spontaneous chemical reactions were those in which heat was evolved in greatest possible quantity. Theoretically, this principle would be strictly true at the absolute zero of temperature. An examination of the fundamental equation, $A - U = T (dA/dT)$, shows that if T vanishes, then A equals U: that is, the change in the free energy of the reaction, which we now know to be the factor which determines whether or not a given change will occur, then equals the total energy change. At this temperature, then, all reactions will be exothermal; while at higher temperatures, such reactions will at first predominate. Moreover, inasmuch as an increase in temperature always increases a reaction velocity,[11] these exothermal

[10] This principle was shown by Le Chatelier to be one particular case of a still wider generalization which has been thus expressed: "If some stress (for example by change of temperature, pressure or concentration) is brought to bear on any system in equilibrium, by which the equilibrium is displaced, the equilibrium is displaced in that direction which tends to undo the effect of the stress (8)."

[11] A very few apparent exceptions to this rule have been noted, explicable on the assumption that negative catalytic effects are involved (9).

reactions are self-sustaining, whereas endothermal reactions are self-limiting, unless they be maintained by a persistently high environmental temperature.

From the van t'Hoff equation another simple deduction of particular interest may be made. If the heat of reaction, U, is zero, then the temperature coefficient of the equilibrium constant will equal zero: that is, $\bar{K}$ will be independent of the temperature. This condition is approximated in the case of most physiological reactions, as has been remarked (10).

The influence of temperature on the velocities of reactions approaching equilibrium cannot be determined in any manner analogous to that by which its influence on the equilibrium itself has been so successfully formulated; for thermodynamical method concerns itself with the description of final states, and not with the progress of phenomena in time. We might nevertheless hope to gain some insight into the nature of this influence by means of the relationship established by the Guldberg-Waage equation: namely, that the equilibrium constant for any reaction is equal to the ratio of the velocity constants of the two opposing reactions which establish the equilibrium. In the terms of the formulation used above, $k_1/k_2 = \bar{K}$. The variability of $\bar{K}$ with change of temperature having been formulated, the problem defines itself as an inquiry into the simultaneous variations of k_1 and k_2 as the reaction approaches equilibrium; since during its course, the velocity at any instant is proportional to the difference between these values. This difference, unlike the corresponding ratio, is directly dependent upon the actual magnitudes of the velocity constants. Consequently, the first necessary step in the inquiry is to define the meaning of these figures.

It is clear, in the first place, that they include all factors of variation that affect the course of the reaction, save the concentrations alone. What are these factors? Primarily, of course, the speed of a reaction in either direction will be proportional to the driving force, to that which we call the chemical affinity between the reacting substances. This, we know, is measurable by the free energy change in the process, which must be considered as determined by the specific characters of the interacting substances, and by these alone. This free energy is known from thermodynamical reasoning to be proportional to $RTln\bar{K}$ (where these symbols have the meanings previously assigned); and it may be measured in a variety of ways, in many cases with precision. But a moment's consideration shows us that the actual velocity must always depend, not only on the intensity of the driving force, but also upon the friction opposed to it, which will vary markedly with the conditions under which the change occurs. Various well-known phenomena, such as those of delayed transition and false equilibrium in general, which comprise many common and familiar instances of the long continued, or even apparently permanent existence of metastable states, indicate the varying and the very great degree in which reaction velocities are thus affected.

Some of these influences are definitely known. The most important is perhaps viscosity, which varies markedly with change of temperature, in a manner which can be empirically formulated. There must be appreciable delay in the speed of reaction, also, whenever molecular orientation must precede combination. Contact catalysis, which is always associated with adsorption, often has a marked influence; and this is largely influenced in turn by other factors of variation—especially in colloidal systems. In such systems, moreover, enzyme catalysis and coagulation—both influenced by change in acidity, basidity and salt concentration in marked degree—may cause similarly undeterminable variation.

These or other influences summarized in the values of the velocity constants modify, in a manner not to be generally formulated, the speed of a chemical reaction, and its variability with change of temture. They may be, of course, in any special case, not beyond the possibility of determination; but their nature is such as to involve many constants characteristic of the interacting substances; while their interdependence, which may be in certain homogeneous equilibria not difficult to define, becomes in general a very complicated matter indeed.

Thus, the velocity of a chemical reaction, far from being a simple function of the temperature, appears upon reflection to be quite the reverse. In view of what has been said, it is not a matter of surprise that the velocities of different reactions range from the immeasurably slow to the immeasurably rapid, and that their accelerations with change of temperature vary widely. Usually this acceleration is very great, much greater than it would be if it increased proportionately with change in the free energy alone. According to the kinetic theory, a rate of chemical combination would be determined by the velocity of molecular movement, which in gases, and probably also in liquids, varies with the square root of the absolute temperature.[12] At ordinary temperatures this acceleration would be about one sixth of one per cent. per degree: the corresponding acceleration of the reaction velocity is, however, seldom observed to be less than five per cent. per degree. This remarkable discrepancy, moreover, is not to be accounted for by the influence of superimposed effects such as change in viscosity, as has been demonstrated conclusively in certain simple cases by careful calculation. It results, therefore, that we must consider the reaction velocity to be yet further influenced, and to a very great degree, by causes not yet considered. In fact, its great acceleration with rise of temperature can be accounted for kinetically only upon the assumption that those molecules alone react which attain a certain high velocity beyond the mean. This indicates, of course, that the phenomena are of a character not to be explained by any such generalizations as the Guldberg-Waage and the van t'Hoff equations; for these, like the thermodynamic laws from which they may be derived, are statistical in their nature, in this sense, that they are based on considerations such as apply to average molecular velocities alone.

Enough has been said to show that for the practical estimation of the influence of temperature on reaction velocities we must at the present time fall back upon rough empirical generalizations based upon the unanalyzed data at our command. It is a singular fact that despite the complexity of the phenomena that condition reaction velocities, their acceleration by change of temperature is not as widely variable as might be expected. "The ratio of velocities for a given temperature interval," says van t'Hoff (11), "usually differs but little from reaction to reaction; and for ten degrees . . . it often lies between two and three. . . . By far the greater number of reactions double or treble their velocity with a ten degree rise of temperature." Illustrating this statement, van t'Hoff tabulates the accelerations of twenty reactions of varied character, the actual velocity constants of which vary between exceedingly wide limits, and finds that the acceleration for a rise of ten degrees shows an extreme variation of 1.2 to 7.14 for all, while for all but three the variation lies between 1.89 and 3.68. This list is supplemented in an interesting way by Euler (12), who tabulates the acceleration coefficients of seventeen enzyme reactions, and finds among these an extreme variation for ten degrees of 1.3 to 5.3. Even this degree of uniformity is remarkable; for a very specific cause of variability affects these latter values, namely, the partial inactivation of the catalyst by heat.

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[12] By definition of temperature (*vide supra*).

The empirical statement of van t'Hoff quoted above is very often referred to, and is commonly used as a rough indication of the degree in which the velocity of a reaction or of a complicated series of reactions will be affected by temperature changes. The more complicated the phenomena, of course, the more likely it will be according to the theory of chance that the rule will hold. It is, however, only very roughly approximate; and, of course, must be discarded altogether if phenomena are under consideration which involve any variable energy interchange between the chemical system studied and its environment. It has, in fact, been the tacit assumption throughout the present discussion that isolated systems alone were under examination. The degree in which van t'Hoff's rule applies to physiological processes is determined first of all by the degree of uniformity in which the partially isolated cell is affected by environmental changes. That it applies at all, is a matter explainable only by the fact that in physiological reactions the amount of total energy change is, excepting for the influence of change in environmental conditions, very small. In consequence, as has been remarked, the equilibrium constant of any such reaction is relatively independent of the temperature. Since this constant is equal in any case to the ratio of the velocity constants, this must mean that these represent nearly the same temperature function; which signifies that in such cases the sum of all forces opposed to that of the change in free energy varies in the same manner with change in temperature. The most influential cause of uniformity in physiological reactions beyond that brought about by small energy change, is to be found in the prevalence of enzymotic catalysis in all such reactions. The contact theory of catalysis requires—and the supposition is supported by many facts—that this influence, in the degree that it operates, reduces all speeds of reaction to the uniform speed of diffusion. This matter cannot be gone into here. It is pertinent only to remark that Euler's values for the temperature coefficients of enzyme reactions for ten degrees are smaller than those summarized by van t'Hoff; which in accordance with theory is what would be anticipated.[13] If, therefore, van t'Hoff's rule be applied to

[13] If all true catalyses are, as seems to be the case, diffusion phenomena, we should in fact expect in such reactions a uniformity of increment with rising temperature much more marked than that noted by Euler. The variability shown by the

physiological reactions, 2 would be more approximate value for this temperature coefficient than 3.

It is much to be regretted that for reasons such as have been touched upon, the chemist is unable to generalize very far concerning temperature and the reaction velocity. Many reactions, and particularly those which occur between the compounds of carbon, exhibit the phenomena of false equilibria. Indeed, it may be said without exaggeration that organic chemistry is the chemistry of metastable states. In the study of such reactions, therefore, the final equilibrium toward which the system tends is a matter of less moment than the relative velocities of change. Particularly do such considerations apply to physiological processes, in which successive reactions overlap and influence each other in a bewildering complexity of delicate adjustments, which from the chemist's viewpoint constitute the essential objective characteristic of the life process. It is in these processes, moreover—which occur within disperse systems incompletely isolated from each other and from the environment, where the continuous phase, probably attenuated to thin laminae, presents very large surfaces of contact—that all phenomena associated with changes in surface energy manifest themselves most markedly. In such systems, which contain not only colloidal complexes but electrolytes in water solution, we must look for the most varied superimposed effects, due not only to false equilibria, but to the varied influences of change in acidity, basidity and salt concentration upon the distribution of solution concentrations at these surfaces; upon the change in the velocity of contact catalysis thus occasioned, or upon that brought about by the presence of characteristic enzymes; and upon alteration in the colloidal substances themselves, which bring about concentration changes in the continuous phase, and readjustments continuously renewed. The picture need not be more completely drawn: no added emphasis need be placed upon the extreme complexity of such phenomena, which appears perhaps even more striking to the chemist's eye than to that of the more accustomed biologist.

In this brief and fragmentary review many interesting and significant facts, all pertinent to the inquiry, have had to be passed over.

coefficients he tabulates must accordingly be interpreted as caused by variable inactivation of the contact surfaces, and corresponding differences in the highly characteristic optima shown by all such reactions. Even in these cases, therefore, which might be expected to show the greatest uniformity in the temperature coefficients, we observe the predominating influence of superimposed effects.

These need not be summarized. It is sufficient if attention shall have been directed to the generalizations of broadest scope and most fundamental implication. The laws of thermodynamics are among the few laws of nature which are precise beyond the limit of accurate measurement. Other generalizations are less precise because of their greater detail of statement, or because of simplifying assumptions deliberately introduced, and are to be looked upon as but first approximations toward the exact expression of the truth. We may even permit ourselves to doubt whether, in terms of formulations similar to those we possess, however more extensive and exact they may become, we shall even approach a complete analysis of physiological phenomena. Already, in the influence of temperature change on reaction velocity we have encountered a phenomenon to which our present generalizations cannot be directly applied. It is by no means absurd to suppose that in colloidal systems which never come to final equilibrium (like these in the living cell) the specific characters of particular regions within the cytoplasm—which cannot by any possibility be homogeneous—will have a determinate effect on the general character of the cell reactions. It would be entertaining to speculate upon the possible connection between many physiological phenomena now little understood and quite possibly dependent upon this circumstance, and individual as contrasted with average molecular velocities. The mere suggestion, however, will suffice to illustrate the point; which seems after all only to imply that present mechanistic conceptions will probably need to be amplified by new considerations, to meet the needs of future inquiry. Our present generalizations, rough though they may be, are none the less of very great value, for they express the implications of demonstrated fact; and the history of scientific theory sustains the hope that in the future they will serve as a secure basis upon which to build the more complete formulations which, by correlating still wider reaches of experience, will yield us deeper insight into these fascinatingly perplexing problems.

Note.—For the purpose of amplifying many of the statements and suggestions made in the course of this brief presentation, the following references may be of value. They have been selected wholly in consideration of their accessibility; and for this reason direct reference to original papers has not been made. Such citations, which will open up practically the whole literature, will be found in the works here listed.

1. For the historical development of the doctrine of energy, see first of all, Merz: History of European Thought in the Nineteenth Century, London, 1903; Vol. II, Chapter 2: "On the Physical View of Nature." This chapter contains very extensive references to the literature. See also, E. Mach: Popular Scientific Lectures, translated by McCormack, Chicago, 1910; Lecture on the Principle of the Conservation of Energy; or, History and Root of the Principle of the Conservation of Energy, translated by Jourdain, Chicago, 1911; also Prinzipien der Wärmetheorie, Leipzig, 1896. See also, P. G. Tait: Lectures on Some Recent Advances in Physical Science, 3d ed., London, 1885. For a complete treatment of the subject, see G. Helm: Die Energetik, nach ihrer geschichtlichen Entwickelung, Leipzig, 1898.

The original memoirs are most accessible in Rumford: Collected Works, London, 1876; Davy: Works, London, 1839; Mayer: Die Mechanik der Wärme, edited by Weyrausch, Stuttgart, 1893; Helmholtz: Ueber der Erhaltung der Kraft, in Ostwald's Klassiker der Exakten Wissenschaften, no. 1; Joule: Scientific Papers, London, 1884; Carnot: Reflexions sur la puissance motrice du feu, Paris, 1824, translated by Thurston, London, 1890; Clausius: Mechanische Wärmetheorie, Braunschweig (1876), 3d ed., 1887–91, translated by Browne, London, 1879. For excerpts translated from the works of Carnot and Clausius, see Magie: The Second Law of Thermodynamics, New York, 1899; and for others, see Ostwald's Klassiker der Exakten Wissenschaften.

With reference to the experimental basis of the fundamental laws see also Preston: The Theory of Heat, London, 1904; Griffiths: The Thermal Measurement of Energy, Cambridge, 1901.

For a consistent presentation of physicochemical phenomena in general from the point of view of energetics, consult Nernst: Theoretical Chemistry, translated by Tizard, London, 1911; and Van t'Hoff: Lectures on Theoretical and Physical Chemistry, translated by Lehfeldt, London, 1898, or the more recent Vorlesungen, Braunschweig, 1901. See also, Mellor: Chemical Statics and Dynamics, London, 1904, especially for a thoroughgoing discussion of speed of reaction and of equilibria.

2. For the development of scientific atomism, see Merz: History of European Thought in the Nineteenth Century, Vol. I, Chapter 5, "On the Atomic View of Nature"; or L. Mabilleau: Histoire de la philosophie atomistique, Paris, 1895, for a more general treatment. See also J. Perrin: Les Atomes, Paris, 4th ed., 1914. For a simple and very clear exposition of the fundamental facts, see Wurtz: The Atomic Theory, translated by Cleminshaw, New York, 1881; and relevant chapters in von Meyer: History of Chemistry, translated by McGowan, London, 1906; or, for a more extensive treatment, Ladenburg: Lectures on the History of the Development of Chemistry since Lavoisier, translated by Dobbin, Edinburgh, 1900.

For excerpts from the original memoirs of Dalton, Avogadro, Gay-Lussac and others, see Alembic Club Reprints, Edinburgh and Chicago, Nos. 2 and 4. See also, for a critical examination of Dalton's original notes, Roscoe and Harden: A New View of the Origin of Dalton's Atomic Theory, London, 1896.

3. See O. E. Meyer: Kinetische Theorie der Gase, Breslau, 1899; or Nernst: Theoretical Chemistry (op. cit.) Book II, Chapter II: "The Kinetic Theory of the Molecule," especially pp. 198–200. See also Perrin: Les Atomes (*op. cit.*) and The Brownian Movement and Molecular Reality, translated by Soddy, London, 1910.

4. For the history of the development of structural chemical formulae, see Ladenburg or von Meyer (*op. cit.*) and for the origin of space formulae and their development, Pasteur: Researches on Molecular Assymmetry (1860), translated as No. 14 of the Alembic Club Reprints (*supra*); van t'Hoff: The Arrangement of Atoms in Space, translated by Eiloart, London, 1898; and G. M. Richardson: The Foundations of Stereochemistry, New York, 1901, a series of translated excerpts from the original papers of Pasteur, van t'Hoff, Le Bel and Wislicenus. See also, Stewart: Stereochemistry, London, 1907. This book gives the present status of the whole subject, and contains a useful bibliography. In Henrich: Neuere Anschauungen über Organische Chemie, Braunschweig, 1908, are brief and readable chapters on the History of Structural Chemistry.

For modern atomistic developments see Perrin (*op. cit.*); Zsigmondy: Colloids and the Ultramicroscope, translated by Alexander, New York, 1909; and in this connection, Hatschek: Introduction to the Physics and Chemistry of Colloids, London, 1913, and relevant chapters in Wolfgang Ostwald: Grundriss der Kolloidchemie, Dresden, 1912.

See also for recent developments in another field, J. J. Thomson: The Discharge of Electricity through Gases, Cambridge, 1903, and The Corpuscular Theory of Matter, New York, 1907; E. E. Fournier d'Albe: The Electron Theory, London, 1909; Rutherford: Radioactivity, Cambridge, 1905, and Radioactive Substances and their Radiations, Cambridge, 1913; for briefer and readable treatments of the same general subjects see especially Righi: The Modern Theory of Physical Phenomena, translated by Trowbridge, New York, 1904; Soddy: The Interpretation of Radium, New York, 1912; J. Cox: Beyond the Atom, New York, 1913.

For a general treatment of the whole subject, see Nernst: Theoretical Chemistry (*op. cit.*), Book II: "Atom and Molecule."

5. Reference is here made to radioactive disintegration, and to certain optical phenomena, notably the specific characters of spectrum lines, their relationship in series and their disturbance by the magnetic field. See, for instance, relevant chapters in Maclaurin: Light, New York, 1909; Righi: (*op. cit.*) and Thomson: The Corpuscular Theory of Matter (*op. cit.*) chapters on the Zeeman effect, or Zeeman: Researches in Magneto Optics, New York, 1913; and Rutherford, and Soddy (*op. cit.*).

6. The data referred to are given in Abderhalden: Textbook of Physiological Chemistry, New York, 1911, p. 335; together with references to Atwater's work and to other previous work in this field.

7. See Richards and Rowe: Proc. Am. Acad. **49**: 173 (1913); especially Table III.

The hydrocarbon data are taken from papers as yet unpublished, on recent work completed in the Harvard laboratories.

8. Quoted from Alexander Smith: Introduction to General Inorganic Chemistry, New York, 1912, p. 260. This book contains the most satisfactory systematic presentation of the fundamentals of present chemical theory.

9. Mellor: Chemical Statics and Dynamics, London, 1904, p. 383. For another discussion of the effect of temperature on chemical reaction, read Chapter XII of this book.

10. For a concise development of the thermodynamical theorems here stated see

Nernst: Theoretical Chemistry (*op. cit.*), Introduction and Book IV: Formulas (I) and (III), pp. 7 to 9; formulas (II) and (IV), pp. 15 to 23; formula (V), pp. 656 to 658; formula (VI), pp. 659, 660.

11. Van t'Hoff: Chemical Dynamics. Vol. I of Lectures (*op. cit.*), pp. 226–228.

12. Euler: General Chemistry of the Enzymes, translated by Pope, New York, 1912; pp. 240, 241.

Columbia University,
New York City.

AN ACCOUNT OF THE CRUCIATE-FLOWERED OENOTHERAS OF THE SUBGENUS ONAGRA.

Harley Harris Bartlett.

The so-called cruciate-flowered Oenotheras are those in which the petals are linear or narrowly oblong instead of broadly obovate. The first one which was described, and the only one, in fact, of the subgenus Onagra which has ever received a distinctive name, was called *Oe. cruciata* by Nuttall, on which account the character of having linear petals has come to be known as cruciateness. The origin of cruciateness is at present a matter of no little interest to geneticists. Some of them hold to the belief, based, it must be said, upon the traditional systematic treatment of *Oenothera*, that all the cruciate-flowered races either belong to *Oe. cruciata* Nutt. or else have acquired the character of cruciateness by hybridization with that species. In this paper the writer records his conclusion that cruciateness has originated independently in several lines of descent and that the aggregate formerly called *Oe. cruciata* must therefore be resolved into several species and varieties. In accord with this conclusion, he has named and defined some of the cruciate-flowered Onagrae which his cultural studies have shown to be constant and distinct.

The original *Oe. cruciata* was found in Massachusetts by Nuttall who sent specimens of it to several botanists. He never described the species himself, and the name was first published as a synonym of *Oe. parviflora* L., by Seringe.[1] The earliest description was that of George Don,[2] who cultivated the plant in 1824 and later characterized it in his General History of the Dichlamydeous Plants, where the name is ascribed to Nuttall. In 1835 Spach[3] described a collective species *Oe. chrysantha*, which included, as varieties, three species of earlier authorship—*Oe. muricata* "Murr." (as *Oe. chrysantha* α: *grandiflora* Spach), *Oe. parviflora* L. (as *Oe. chrysantha* β: *parviflora* Spach) and *Oe. cruciata* Nutt. (as *Oe. chrysantha* γ: *cruciata* Spach). Torrey and Gray[4] likewise treated *Oe. cruciata* as a variety, but whereas Spach had reduced the allies of *Oe. biennis* to two species, these authors ranged them all as varieties under *Oe. biennis*. The names *Oe. biennis* var. *cruciata* T. & G. and *Oe. cruciata* Nutt. are strict synonyms.

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[1] Seringe, in D. C. Prod. **3**: 47. 1828. "*Oe. cruciata* Nutt. in litt. ex herb. DC. et Mercier."

[2] Don, G. Gen. Hist. Dichlam. Pl. **2**: 686. 1832. "*Oe. cruciata* (Nutt. mss.) stem reddish, rather hairy; leaves lanceolate, acuminated, denticulated, glabrous but the upper ones are rather downy; flowers sessile; petals linear, rather shorter than the anthers; calycine segments reflexed, linear, mucronate, longer than the petals, but about equal in length to the stamens; lobes of stigma thick, conniving, or spreading a little; capsule cylindrical, hairy. ♂ H. Native of North America. Flowers small, yellow. Cross-petalled Evening Primrose. Fl. July, Aug. Clt. 1824. Pl. 2 to 4 feet."

Oenothera biennis var. *cruciata* "with singularly small and narrow linear-oblong petals, and smooth pods" ran through all the editions of Gray's Manual, from the first to the fifth, with no specific statement of geographic range. In the sixth edition, however, we find the statement that "var. *cruciata* Torr. & Gray, with small narrow petals, appears to be merely a rare garden (?) sport. E. Mass." The change appears to have been made because no wild specimens of *Oe. cruciata* had ever been collected, or at any rate none had been preserved in public herbaria, between the time of Nuttall's original discovery of the species (probably 1822 or 1823, soon after his arrival in Cambridge), and 1889, a period of more than sixty years. Cultivated specimens, collected in the Harvard Botanic Garden in 1856 and 1875, were the only material of the species known to Watson, the editor of the sixth edition of Gray's Manual and a monographer of the genus Oenothera. His surmise that *Oe. biennis* var. *cruciata* might have originated as a garden sport shows clearly that until comparatively recent years such a form has had only a traditional status as a wild plant in this country.

We have seen that Don cultivated the true *Oe. cruciata* in 1824, and there is no intrinsic improbability but that the form which is still found in European botanic gardens is genetically related to it. It is more likely that the form cultivated at the Harvard Botanic Garden in 1856 and 1875 was a reintroduction from Europe than that it had been continuously in cultivation since Nuttall's time. Of course, it may have had an independent origin. Since the variability of the petals in the European garden form shows that it has been hybridized with some broad-petaled species, and cannot therefore be considered typical of *Oe. cruciata*, de Vries [5] has named it *Oe. cruciata* var. *varia*. He used this nominal variety for the crosses between "*Oe. cruciata*" and other species which are described in the second volume of Die Mutationstheorie. Because of its unknown, mixed ancestry it was an undesirable type for use for further experiments. De Vries, therefore, wrote to this country for seeds of the true *Oe. cruciata*, which he had no reason to suspect was not well known here, for Small[6] had only recently (1896) restored it to specific rank, under the name *Onagra cruciata*, and had cited it not only from Massachusetts but also from Vermont. The Vermont record was probably based upon specimens collected by Grout at Brattleboro and Vernon; the localities were later published in the Flora of Vermont.[7]

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[3] Spach, E. Monographia Onagrearum. Nouv. Ann. Mus. 4: 355. 1835.

[4] Torrey and Gray, Fl. N. Am. 1: 492. 1840. "*Oe. biennis* (Linn.) ϵ. *cruciata*: petals (abortive) linear-oblong, shorter than the stamens; tube of the calyx 2–3 times the length of the segments; capsules nearly glabrous.—*Oe. cruciata Nutt.! in DC. l.c.* (under *Oe. parviflora.*)"

In response to his request, de Vries received seeds and rosettes from MacDougal, collected at Sandy Hill, New York, near Lake George, and seeds from Robinson, collected at Jaffrey, New Hampshire—all supposedly *Oe. cruciata*. These were grown at Amsterdam in 1903. The material from Sandy Hill included two very distinct types, both of which were found among the plants from seeds as well as among those from rosettes. The differences between the types were slight, but striking and absolutely without transition. Most of the individuals had rather broadly linear petals and comparatively thick flower-buds. The rest had narrowly linear petals and slender flower-buds. Robinson's seeds from Jaffrey gave a third type, diverging from both of the others in its much longer calyx tube and more slender stem, inflorescences and foliage. De Vries[8] interpreted his findings as follows: "It seems not improbable that *Oenothera cruciata* includes a group of lesser unities, and may prove to comprise a swarm of elementary species, while the original strain might even now be in a condition of mutability." In regard to the two types from Sandy Hill, he said: "Probably two elementary species were intermixed here, but whether one is the systematic type and the other a mutation remains to be seen."

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[5] De Vries, H. On Atavistic Variation in *Oenothera cruciata*. Bull. Torr. Bot. Club **30**: 75–82. 1903. *Also* Die Mutationstheorie. **2**: 100. 1903.

[6] Small, J. K. Oenothera and its Segregates. Bull. Torr. Bot. Club **23**: 169. 1896.

[7] Brainerd, Jones and Eggleston. Flora of Vermont, 1900, p. 63.

[8] De Vries, H. Species and Varieties, 1905, p. 590. See also letter quoted by MacDougal, Vail, Shull and Small, Mutants and Hybrids of the Oenotheras, 1905, p. 12.

MacDougal[9] has stated definitely that the New Hampshire type appeared as a mutation from one of the Lake George types in his cultures of 1903 and 1904, at the New York Botanical Garden. His statement follows: "The cultures of 1904 included over sixty specimens of *O. cruciata* which reached the adult stage, and included not only the two forms which he [de Vries] had observed to arise from the seed and roots sent him from this place, but also the third obtained only from material from New Hampshire. It is obvious, therefore, that one form arises spontaneously from one of the other two forms suddenly, and dried specimens from the crop of 1903 in the New York Botanical Garden show that it originated in this manner here in the first year of cultivation, although the second half of the same lot of seeds sent to Professor de Vries failed to give rise to it in Amsterdam" (*l. c.*, p. 13). "*O. cruciata* as it exists at the present time in the cultures of the New York Botanical Garden and in the Botanical Garden of Amsterdam is composed of three elementary species, which are fairly distinct and without intergrading forms. A careful analysis of the occurrence of the group leads to the inevitable conclusion that one of the forms is in a mutating condition" (*l. c.*, p. 52).

MacDougal defined and figured his three elementary species of "*Oe. cruciata*" but quite unaccountably failed to say whether his cultures of 1904 were grown from garden seeds of the previous year or from more of the original wild seed which he had planted in 1903. If the 1904 cultures were a second generation, it stands to reason that he would have known which of the Lake George types was in a mutating condition, and would not have written, referring to the origin of the third form, "The evidence at hand, therefore, seems to confirm the suggestion as to the mutability of the species, but nothing may be said as to which of the types constitutes the parent" (*l. c.*, p. 13). If, on the contrary, the 1904 cultures were from wild seed, MacDougal had no evidence whatever that one of the Lake George types was "in a mutating condition." On either supposition as to the source of the 1904 seed, his conclusion, far from being "inevitable" is not even plausible.

The original seed collection from Sandy Hill was doubtless gathered from individuals of two different species, growing at the same locality, as is so frequently the case with closely related species of the subgenus Onagra. This explanation of the source of the two species which De Vries differentiated at Amsterdam is amply verified by the fact that wild rosettes from the same locality as the seeds proved to belong to the same two species. If it were not for a suspicion that two of MacDougal's types were in reality non-hereditary seasonal phases of the same species, one might almost as readily believe that there were three species in the mixed seed collection as that there were two, and thus explain the supposed mutation. MacDougal said of the third type that all the individuals of it completed their seasonal development much earlier than the other two. It seems not impossible that the set of plants which developed early in the season may have appeared so different from later plants of the same form as to be mistaken for a mutation. Several times in the writer's experience with the Oenotheras, a culture has consisted of two types of plants, those which went through the life-cycle of a biennial, forming a dense rosette which persisted for weeks, even though the plants, by virtue of being started early in the spring under glass, completed their development in one season, and those which went through a strictly annual life-cycle, forming only a transitory rosette of a few leaves before they become cauline. Since the annual type is lower and less robust, due probably to its not having elaborated a reserve food supply for use during the period of rapid growth, one might easily be misled into interpreting it as a mutation.

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[9] MacDougal, assisted by Vail, Shull and Small. Mutants and Hybrids of the Oenotheras, 1905.

After MacDougal gave up his work on Oenothera, his cultures were continued by Dr. Geo. Harrison Shull. Shull, however, cannot supply seeds of the questionable third form of "*Oe. cruciata*" and writes that he has never cultivated it. Of three lots of seed which were originally turned over to him, two yielded the same type.

Even if we assume that MacDougal's third form was really distinct, there is no reason to believe that he correctly identified it with Doctor Robinson's New Hampshire type. De Vries characterized the latter as having a strikingly longer hypanthium than either of the Lake George types, whereas MacDougal figured his dubious third type as having a shorter hypanthium than one of the Lake George forms. Moreover, it appears that MacDougal never cultivated the New Hampshire type, but made his identification merely from de Vries's very brief account of it.

The writer believes that the two Sandy Hill types (and probably also the New Hampshire type) are distinct taxonomic units which for the present at least must be treated as species. Neither of them is the same as *Oe. cruciata* Nutt. There is a possibility that they are the cruciate-flowered varieties of thus-far undescribed broad-petaled species, and that further collections may discover their true relationships in this direction. The cruciate character has probably arisen many times in totally unrelated strains, so there is no reason to believe that our three types are of necessity derivatives of any common parent species, even if they are true species in the sense that cruciateness is only one of a group of correlated characters by which each differs from its nearest ally.

During the summer of 1913, the writer had the two Vermont strains in cultivation from seeds furnished by Professor de Vries, but not the New Hampshire strain. The latter has not been grown recently at Amsterdam. As a matter of fact, de Vries has continuously maintained only one of the Sandy Hill types in his cultures; the seeds of the other which he sent were old and germinated poorly, although a sufficient number of plants were obtained for a taxonomic study. It turned out that neither of the Vermont types corresponded with the original *Oe. cruciata* of Nuttall, the type specimen of which is preserved in the Candollean Herbarium. Through the kindness of M. Cas. de Candolle a photograph of this specimen was obtained, which is reproduced as figure 1. There is also one of Nuttall's original specimens in the Herbarium of the Philadelphia Academy of Natural Sciences, which the writer has carefully compared with the types sent by de Vries. Not only are the latter distinct from *Oe. cruciata*, but in the writer's opinion they are specifically distinct from one another.

After the writer had reached this conclusion, and had planned to publish the two Lake George types as new species, he learned, quite by accident, that Shull had likewise assigned names to them, which he would soon have published in connection with a discussion of certain genetic experiments. In order not to cause Shull any inconvenience, the writer proposed that the species be published with technical diagnoses in this paper, but under the names which Shull had provisionally given them and had attached to a great many specimens and permanent photographic records. It is with Shull's approval that the names *Oe. atrovirens* and *Oe. venosa* are here proposed under our joint authority. In justice to Doctor Shull, however, it is only fair to state that he does not altogether agree with the writer as to the specific independence of the two forms. Thus, under date of September 26, 1913, he writes: ". . . I must confess that I prefer to consider them as subspecies of *Oe. cruciata* Nutt. and would have published them as such if left to my own initiative. But of course if you are consistently describing everything which is genotypically distinct and morphologically distinguishable as species, these forms certainly deserve the same treatment."

The so-called *Oe. cruciata* of recent collectors, of which numerous specimens have accumulated in our herbaria since 1890, seems to consist not of one species or group of genetically related species, but of the cruciate varieties of several independent broad-petaled species, together with a number of species in which the cruciate character has probably become specific. In other words, *Oe. cruciata, sensu latiore*, is a purely artificial group, the components of which must ultimately be distributed according to their true relationships. The writer believes that cruciate variations of any species of Onagra are likely to occur as bud sports or germinal mutations. The almost certain origin of the cruciate variety of true *Oe. biennis* on the sand dunes of Holland is a case in support of this belief. One solitary individual of this variety was found by Ernst de Vries in August, 1900, growing with the typical form on the dunes near Santpoort. No such form had hitherto been known. As de Vries[10] pointed out, it could not have originated by hybridization with any other cruciate form, since there was no such form in Holland at the time except the rare *Oe. cruciata* var. *varia* of botanic gardens. Quite aside from the extreme improbability that two normally self-pollinated strains growing at a great distance should have been crossed, it will suffice to point out that *Oe. cruciata* var. *varia* de Vries, is characterized by a dark red coloration of the stems and foliage which is dominant in all its hybrids. In the cruciate variety of *Oe. biennis* there is no such coloration. The writer's cultures of 1913 included fifty plants of it, from seeds sent by de Vries, descended from the original plant found by his son. They were a uniform lot, and differed in no respect from *Oe. biennis, sensu strictiore*, other than that the petals were linear instead of obovate. The variety can, therefore, hardly be regarded as other than a mutation, the occurrence of which gives a tangible clue to the manner in which other cruciate forms have arisen.

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[10] De Vries, H. Die Mutationstheorie, 2, 1903, p. 599.

During the past summer the writer grew fifty plants of an undetermined Oenothera from Springfield, Missouri, the seeds of which were collected from one individual by Mr. P. C. Standley. All were strictly uniform except for one plant, of which a single branch bore cruciate instead of broad-petaled flowers. The petals of the cruciate flowers would be characterized as narrowly oblong rather than linear, thus contrasting strongly with those of the cruciate variety of *Oe. biennis*, for instance, but nevertheless strikingly divergent from the petals of normal flowers. Doctor Shull suggests in a recent letter that his experience would lead him to suspect that the occurrence of such a bud variation indicated recent hybridization with a cruciate strain. In this connection de Vries's experience with *Oe. cruciata* var. *varia* is of interest. He found that this variety, in which the petals are inconstant in form, segregated roughly into three groups, a purely cruciate-flowered, an intermediate and an atavistic (broad-petaled) group. Except for rare cases of bud variations which showed a return to the cruciate type, the progeny of the broad-petaled group were constant. Although it is by no means impossible that the broad-petaled Springfield strain is a segregate from a hybrid between a broad petaled and a cruciate strain, the likelihood of such an origin is not great. Thus far no cruciate-flowered Oenothera is known from Missouri, and none has been collected nearer than Mobile, Alabama, or Washington, D. C. Although the cruciate bud variation of the Springfield strain has not yet been tested for constancy in a second generation, it seems to be of sufficient interest to justify calling attention to it in connection with a discussion of the origin and relationships of the cruciate types.

Davis[11] has recently published an adverse criticism of a paper by Stomps[12] on mutation in the Dutch *Oe. biennis*, which is based entirely upon a misconception of the relationships of the cruciate Oenothera of the sand dunes of Holland. It was perhaps unfortunate that de Vries named this plant *Oe. biennis* var. *cruciata*, in view of the fact that the same name had been formerly used by Torrey and Gray for *Oe. cruciata* Nutt., and may even again be taken up as the valid name for the older type if ideas as to the limits of species continue to shift. Davis made the error of regarding de Vries's homonym as a synonym of *Oe. cruciata*

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[11] Davis, B. M. Mutations in *Oenothera biennis* L.? Am. Nat. **47**: 116–120, 1913.

[12] Stomps, T. J. Mutation bei *Oenothera biennis* L. Biol. Centralb. **32**: 521. 1912.

Nutt., which was treated in American floras for decades as *Oe. biennis* var. *cruciata* T. &. G. De Vries evidently anticipated that no confusion would result from the use of the name *Oe. biennis* var. *cruciata* a second time, because of the fact that he was recognizing the former var. *cruciata* as a species. That misunderstanding has arisen is clear from the following extracts from Davis' article: ". . . we find that the so-called 'mutants' were not derived from the pure Dutch *biennis* of the sand dunes but from a cross between this race and a type designated *O. biennis cruciata.* This fact seems to the writer of fundamental importance in judging the conclusions of Stomps. It should be made clear that the form '*O. biennis cruciata*' is recognized in the more recent taxonomic treatments as a true species sharply distinguished from types of *biennis* by its floral characters. Whatever may have been the origin of *O. cruciata* or its possible relationship to *O. biennis*, a cross between these types must certainly be regarded as a cross between two very distinct evolutionary lines and its product a hybrid in which marked modifications of germinal constitution are to be expected. . . . Stomps assumes that the *cruciata* in Holland is a mutant from the Dutch *biennis*, but his belief rests upon no direct evidence. *Cruciata* has never appeared in the extensive cultures of the Dutch *biennis* grown by de Vries and Stomps. Neither have we any direct evidence that the American *cruciata* has come from any form of *biennis.* It is true that the species *cruciata* and *biennis* appear to be closely related, but it is equally clear that they constitute very distinct lines each with a long period of evolutionary independence. I cannot see the justification for Stomps's attitude when he treats a cross between the *biennis* and *cruciata* of the sand dunes of Holland as though it were the combination of forms within the same species which have similar germinal constitutions. Stomps lays emphasis on the purity of his material of *biennis* and *cruciata* which had been carried along for several years in pure lines from original wild plants of the sand dunes. He states that the crossing of these two forms is concerned alone with the floral peculiarities of *cruciata*, since in all other characters the two types are the same. It seems to the writer hardly possible that lines so well established as *biennis* and *cruciata* can be absolutely the same in all respects except that of flower form, although this is obviously the most important point of difference. The American forms of *cruciata* are exhibiting among themselves remarkable differences of germinal constitution."

As already stated, the writer has cultivated *Oe. biennis* var.

cruciata de Vries, and has found it to be, as de Vries and Stomps have said, quite indistinguishable, except as to floral characters, from the true Dutch *Oe. biennis*. It is quite impossible that it has any relationship with the older *Oe. cruciata* Nutt. That it has originated as a mutation from *Oe. biennis* not only once, but several times, independently, seems the most likely explanation of its sporadic occurrence. In his latest book, de Vries[14] states that he has received living specimens from Prof. H. Klebahn, collected on the Lüneburg heath. Its presence in Holland at several localities besides Sandpoort has also been reported to him. Although rare, it seems to be of rather wide distribution, and decidedly worthy of taxonomic recognition. Since the name *Oe. biennis* var. *cruciata* is preoccupied, and therefore likely to continue to be a source of confusion, the writer proposes to substitute for it the new designation *Oe. biennis* var. *leptomeres*.

Oenothera cruciata of the American Manuals has been reported only from New England and northern New York. It is represented in the Gray Herbarium by specimens from Nova Scotia (Sable Island, *Macoun*); Maine (Madison, Lexington and Cornish, *Fernald*; Cumberland, *Chamberlain* and *Knowlton*): New Hampshire (Surry, *Fernald*; Rollinsford, *Parlin*; Dublin, *Robinson*): Vermont (Brattleboro, *Grout*; Vernon, *Robinson*): New York (Axton, Adirondack Mts., *Rowlee, Wiegand* and *Hastings*): and Massachusetts (Rockport, Cape Ann, *Bartram*; Northampton, *Robinson*). Seeds from these or any other localities would be very welcome to the writer of this article. Doctor Robinson has again been so kind as to collect seeds of a cruciate Onagra at Jaffrey, New Hampshire, which will be grown during the coming summer. There is little doubt that careful cultural study will bring other cruciate strains to light from the region of New England and northern New York, in addition to *Oe. cruciata* (*sensu strictiore*), *Oe. atrovirens* and *Oe. venosa*.

Although no cruciate Onagrae have hitherto been reported from the region south of New England, Doctor Shull has had an undescribed species from Long Island in cultivation for several years. It will be treated in a future paper. The writer has cultivated two others, one from Mobile, Alabama, which must be studied another season before it can be described, and a second from Montgomery County, Maryland, to which the name *Oe. stenomeres* is here given.[15]

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[14] De Vries, H. Gruppenweise Artbildung, 1913, p. 298.

[15] Since this article was submitted for publication Bicknell (Bull. Torr. Bot.

Oenothera stenomeres has now been under observation through four generations. The starting point of the pure line was a half grown cauline annual plant which was transplanted to the writer's Bethesda garden from Chevy Chase, Maryland, a suburb of Washington, in midsummer of 1910. It was not known to be a cruciate plant until it flowered, but cruciate plants had been seen in the same general locality in 1909, and have been observed frequently every summer since then both at Bethesda and Chevy Chase. Ten plants were brought to maturity in 1911, 16 in 1912, and 106 in 1913. Not until the last year did the strain show any noteworthy variation, although, to be sure, the cultures were very small. In the fourth generation, however, there were at least three mutations in the culture of 106 plants. One was a practically self-sterile plant, otherwise indistinguishable from the type, the second had very large, thick buds and short, thick fruits, and the third was a remarkable plant which the writer is inclined to consider as perhaps the most striking example of mutation which has thus far been reported in any species of Oenothera except *Oe. Lamarckiana*. This plant was unusually stout and hairy. About midsummer the growth of the main stem and of all the branches but one was stopped by the development of a large rosette, in every way like the winter rosettes of the biennial strains, at the end of each stem. In this condition the plant remained through the rest of the summer and fall, except that the exceptional branch flowered normally. The form proved to be completely fertile to its own pollen and formed large, well-filled capsules. The flower buds were densely pubescent, which is not the case in the type, but the most remarkable character of the plant was that the petals also were densely hairy. All of the cruciate Onagrae appear to have a few scattered hairs on the petals (a microscope is often necessary for their detection) but the petals of this plant were so thickly covered with long appressed hairs that they appeared canescent in the buds, a day or two before flowering time, when they had not yet reached full size. Although larger, the petals of the mutation were narrow, as in all the other plants of the species, and were yellow at maturity.

A further discussion of this very interesting mutation is reserved until its seeds shall have been grown. For the present it will suffice

Club 41: 79. 1914) has published *Oenothera stenopetala*, a cruciate-flowered ally of *Oe. Oakesiana*, from the island of Nantucket. It seems to be clearly distinct from any of the species treated here.

to point out (1) that it occurred in the fourth guarded generation of the strain, (2) that the species is strictly self-pollinating because the stamens surround the stigmas and because the flowers generally open only imperfectly or not at all, (3) that any accidental crossing with a broad-petaled species would have been evident in the next generation, (4) that until the year when the mutation was found there had been no other cruciate strain in the garden or vicinity with which it could have accidentally crossed, (5) that the densely hairy petals constituted an absolutely discontinuous variation, for in all the other plants of the strain they were glabrous except under microscopic examination, and (6) that the new character could not have been introduced into the strain by hybridization, inasmuch as no other Oenothera with which it could have crossed has hairy petals.[16] The new mutation may be called **Oe. stenopetala** mut. **lasiopetala.**[17]

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[16] De Vries (Bull. Torr. Bot. Club **30**: 75–82. 1903) reports considerable variation in the degree of pubescence of the petals of *Oe. cruciata* v. *varia.* This form is of garden origin and is known only in European botanic gardens. In America there seems to be no Onagra of which the petals appear other than glabrous to the naked eye.

[17] The writer proposes that a trinomial system of nomenclature be used for mutations of garden origin, in order to set them clearly apart from forms of which cognizance must be taken in floras. Gates has made the suggestion that mutations be indicated by placing the abbreviation "mut." between the terms of the binomial. According to his practice, for example, the name of *Oe. lata,* the well-known mutation from *Oe. Lamarckiana,* would be written *Oe.* mut. *lata.* The use of a trinomial system, however, would appear to have decided advantages over a binomial system. Gates, for instance, has reported the discovery of a mutation from *Oe. biennis* parallel to *Oe. lata,* which he has called *Oe. biennis* mut. *lata* in order that the name might show the parallelism of the two variations. Certainly it is very helpful to be able to use the same mutational designation in such cases, and a trinomial system makes it possible to so do consistently. The use of the trinomial for all mutations would not imply anything about the degree of differentiation of any particular mutation; in other words, the category *mutatio* would indicate manner of origin rather than kind of variation. *Oe. gigas* de Vries is a mutation of specific rank; probably most botanists would consider *Oe. brevistylis* to be of varietal rank. If it were considered desirable, the abbreviations "mut. sp." and "mut. v." (*mutatio specifica*; *mutatio varietalis*) might be applied as follows: *Oe. Lamarckiana* mut. sp. *gigas* and *Oe. Lamarckiana* mut. v. *brevistylis.* The latter variation is found wild in Holland; if it were necessary to include it in a flora the name would be *Oe. Lamarckiana* var. *brevistylis.* The former has been introduced into this country; if it spreads and must be taken account of by systematists the name would stand as *Oe. gigas.*

KEY TO CRUCIATE ONAGRAE DISCUSSED IN THIS PAPER

- Free sepal tips distinctly infraterminal, *i. e.*, separated in bud.
  - Free sepal tips very densely pubescent with appressed, sharp hairs. *Oe. cruciata.*
  - Free sepal tips thinly pubescent, not markedly more so than the upper part of the bud.
    - Main stem and basal branches with numerous lateral flowering branches. Hairs of calyx segments below free tip of two types, cylindrical and acute. *Oe. venosa.*
    - Main stem and basal branches simple below the inflorescence. Hairs of calyx segments below free tip all cylindrical. *Oe. atrovirens.*
- Free sepal tips terminal, *i. e.*, appressed to one another at the base in bud.
  - Main stem bearing either a flowering branch or a flower in every axil. *Oe. biennis.* var. *leptomeres.*
  - Main stem with a few flowering branches below the terminal spike, which pass gradually into abbreviated non-flowering branches toward the middle of the stem. *Oe. stenomeres.*

OENOTHERA CRUCIATA Nutt.

The specimen in Herb. Phil. Acad. appears to be identical with the specimen in Herb. de Candolle (v. figure 1). It consists of the terminal part of a large branch (or main stem?) bearing one flowering branch in the lowest axil. Stem red on side exposed to the sun, otherwise green, with pubescence of two types,—long spreading or ascending sharp hairs mostly red-tuberculate at base and a thin scarcely visible indument of short, acute, crispate hairs. Upper leaves lanceolate, distantly repand-denticulate, broadest near the base and tapering to the acute or acuminate apex, somewhat pubescent on both sides with appressed sharp hairs. Bracts of the inflorescence lanceolate, broadest near the middle, upper ones with some hairs of the cylindrical, thin-walled, round pointed type. Hypanthium about 33 mm. long, very slender, densely puberulent with cylindrical hairs and sparsely pilose with long, sharp hairs. Calyx segments 10–11 mm. long, puberulence more sparse than on hypanthium and pilosity more dense: free tips about 2 mm. long, densely appressed-pilose, distinctly infra-terminal and well separated in bud. Buds somewhat clavate. Ovary 7–8 mm. long, with both the short, cylindrical and long, sharp, hair types.—Massachusetts, Nuttall, 1825.

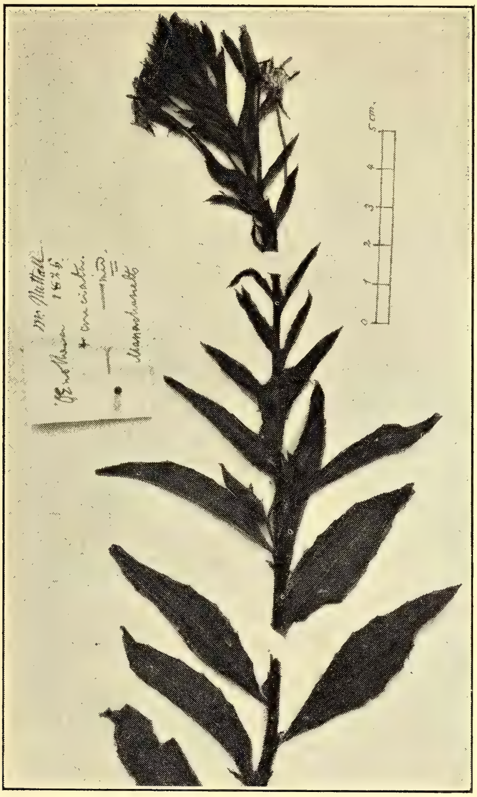


FIG. 1. *Oenothera cruciata* Nutt. Photograph of the type specimen at Geneva, sent by M. Cas. de Candolle.

Oenothera atrovirens Shull & Bartlett, sp. nov.

Biennis. Rosula matura 3.5–4 dm. diametro, altero anno caulem proprium ramosque radicalis simul emittens, foliis spatulatis atroviridibus acutis, maximis ca. 20 cm. longis, 3–4 cm. latis, secus costam paululum bullatis, ad basin versus minime profunde et distanter sinuato-dentatis. Planta matura ca. 9 dm. alta ex caule proprio semper obliquo ramisque ca. 12 multum brevioribus radicalibus patentibus constans, utrisque superiore parte subnutantibus. Caulis proprius infra inflorescentiam simplex, solum ramulos foliis breviores nunquam florentes gerens; inflorescentia vel ex spicis pluribus brevibus densissimis composita vel raro simplex. Rami radicales cauli similes sed brevioribus ramulis axillaribus plerumque parvissimis rosulatis instructi; inflorescentiae saepissime simplices. Caules in latere ad solem spectante rubri, altero latere virides vel coloribus duobus sparsi, pilis triformibus tenuiter vestiti, I paucissimis longis acutis basi rubrotuberculatis, II multis minutis subappressis valde verrucosis, apicem versus angustatis sed obtusiusculis, III aliisque minutissimis laevibus ampulliformibus. Folia atroviridia subnitida, pendula vel reflexa, anguste lanceolata sinuato-denticulata, maxima ca. 12 cm. longa, 1.5 cm. lata, mediocria ca. 6 cm. longa, 1 cm. lata, tenuissime puberula, utrinque pilis brevibus acutis subverrucosis, supra aliis etiam minutis ampulliformibus commixtis. Bracteae inferiores ovato-lanceolatae foliaceae floribus breviores, superiores juventate ovariis aetate fructibus paulum longiores, lanceolatae acuminatae apice rubrae, utrinque fere glabrae sed paucissimis pilis ampulliformibus praeditae, margine pilis subulatis laevibus acutis basi dilatatis eis foliorum longioribus dense ciliatae. Hypanthium 25–34 mm. longum 2.2 mm. crassum apicem versus abrupte expansum, sparse viscido-pubescens, pilis pluribus cylindricis apice rotundatis et paucis multo

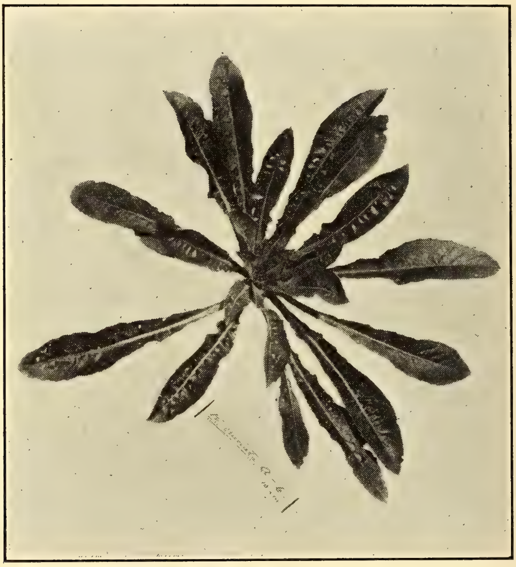


FIG. 2. *Oenothera atrovirens* Shull & Bartlett. Mature rosette. (The label is 10 cm. long.)

brevioribus ampulliformibus. Calycis segmenta ca. 14 mm. longa inferiore parte pilis cylindicis vestita, apicibus liberis 3.5–4 mm. longis infraterminalibus pilis subulatis eis bractearum marginis similibus praeditis. Petala oblonga 8–9 mm. longa, 2.5 mm. lata, supra glabra, subtus pilis sparsis ornata, aliis laevibus acutis cum aliis cylindricis intermixtis. Ovarium 11–12 mm. longum fere glabrum, pilis sparsissimis acutis laevibus longitudine valde variantibus aliisque minutissimis ampulliformibus vestitum, ad anthesin ex rhachi recte divergens. Fructus maturi densissime aggregati ascendentes, saepe 30 mm. longi, fere teretes, prope basin ca. 7 mm. crassi, sursum angustati, apicibus liberis brevibus emarginatis.—Seeds collected near Sandy Hill (now Hudson Falls), New York, 1902, distributed by D. T. MacDougal and now cultivated in several gardens. Type (from a cultivated plant) *Bartlett* 3500, in U. S. Nat. Herb., nos. 693736–7. This is the "*Oe. cruciata*" of de Vries's Gruppenweise Artbildung.

Oenothera venosa Shull & Bartlett, sp. nov.

Biennis. Rosula matura ei *Oe. atrovirentis* valde similis, sed colore aliquantum pallidior. Planta matura 7–9 dm. alta ramosissima. Caulis proprius infra spicam terminalem multos ramos floriferos saepe usque ad basin ferens; rami radicales quoque ramosi nunquam simplices. Caules maculosi; pilis paucissimis basi rubrotuberculatis, multis similibus verrucosis longitudine valde variantibus absque tuberculo rubro ad basin, et sparsis ampulliformibus. Foliorum pubescentia ut in *Oe. atrovirenti*; margo argutius dentata; positio vel patens vel modice reflexa, nec pendula. Bracteae foliaceae non deciduae eis *Oe. atrovirentis* ampliores, inferiores interdum fructibus bis terve longiores, apice rubrae, in superiore pagina margineque pilis aliis acutis subverrucosis aliis vel subclavatis vel cylindricis apice rotundatis sparse tectae, subtus pilis uniformibus acutis. Hypanthium 26–38 mm. longum, ca. 1.8 mm. crassum; pilis biformibus aut acutis sublaevibus aut cylindricis apice rotundatis. Calycis segmenta ca. 20 mm. longa, pilis paucis eis hypanthii similibus praedita; appendicibus liberis 5–5.5 mm. longis conspicue infraterminalibus apice rubris. Petala lineari-oblonga ca. 9–10 mm. longa, 1.8 mm. lata, solum supra pilis sparsis cylindricis vestita. Ovarium pubescens, pilis intermixtis, aliis acutis laevibus cum aliis cylindricis. Fructus quam in *Oe. atrovirenti* distantiores, ca. 25 mm. longi, fere teretes, prope basin 6.5

mm. crassi, modice pubescentes, pilis conspicuioribus ascendentibus acutis.—Seeds collected near Sandy Hill (now Hudson Falls), New York, 1902, distributed by D. T. MacDougal. Type (from a cultivated plant) *Bartlett* 3501, in U. S. Nat. Herb. nos. (6) 393738–40.

error

Oenothera biennis var. **leptomeres** Bartlett nom. nov.

Oenothera biennis var. *cruciata* de Vries *non* T. & G.

A speciei forma typica differt solum petalis lineari-oblongis.—The cultivated line originated from seeds of a plant discovered at Santpoort, Holland, *Ernst de Vries*; known from other localities in Holland and from Lüneberg, Germany, *Klebahn.*

Oenothera stenomeres Bartlett, sp. nov.

Annua. Rosula ex paucis (ca. 10–14) foliis constans, maximis ca. 20–22 cm. longis, 4–4.5 cm. latis, acutis vel obtusiusculis, infra mediam valde sinuato-dentatis, cito caulem et interdum 3–5 ramos radicalis emittens. Planta matura 10–12 dm. alta. Caulis proprius inter mediam spicamque terminalem ramos floriferos ferens, superiores ac longiores primo florentes, demum inferiores ac breviores quos plerumque in ramulos brevis non florentes gradatos sunt. Rami numerosi plantae partis inferioris cauli proprio omnino similes sed breviores, nec in ramos superioris partis transgradientes. Caules rubri et virides, pilis longis acutis rubrotuberculatis sparsi et aliis similibus sine tuberculo rubro, longitudine variantibus, plerumque minutis, pubescentes. Folia ovatolanceolata in medio caule ca. 15 cm. longa, 4 cm. lata, utrinque acuta, modice sinuato-dentata, pilis acutis vestita. Bracteae lanceolatae fructibus et ovariis tertia parte longiores, tenuiter pubescentes, margine pilis aliis acutis verrucosis, aliis ampulliformibus ciliatae. Hypanthium ca. 35 mm. longum, 1.5–1.8 mm. crassum, pilis paucis acutis arcuatis praeditum. Calycis segmenta ca. 14 mm. longa pilis eis hypanthii similibus praecipue supra mediam sparse vestita; apicibus liberis terminalibus, 2.5 mm. longis dense pilosis, saepissime (in floribus cleistogamis vel subcleistogamis) basi persistenter adnatis. Petala lineari-oblonga, 10–11 mm. longa, 2 mm. lata, extus prope apicem pilis paucis subulatis acutis ornata, intus glabra. Stamina stigmata aequantia. Ovarium ca. 12 mm. longum, etiam pilis acutis solis indutum. Fructus modice congesti, ca. 2.2 cm. longi, sparse pilosi, apicibus liberis brevibus truncatis.—Chevy Chase and

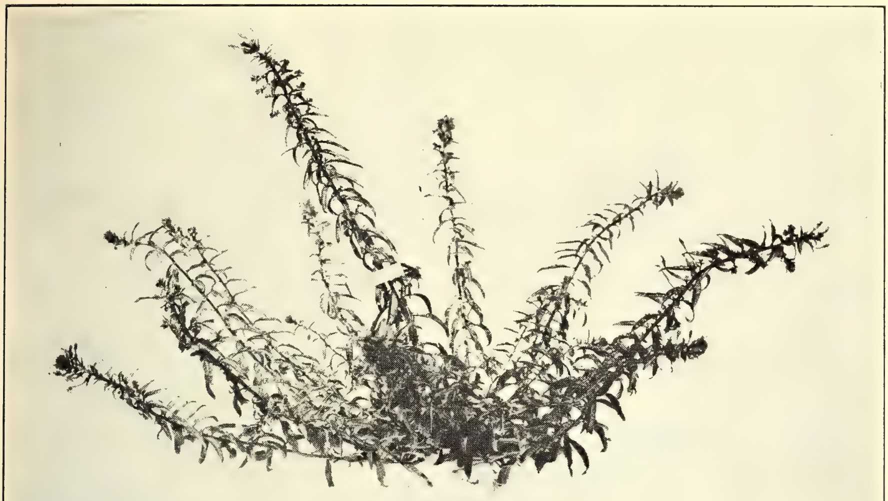


Fig. 1

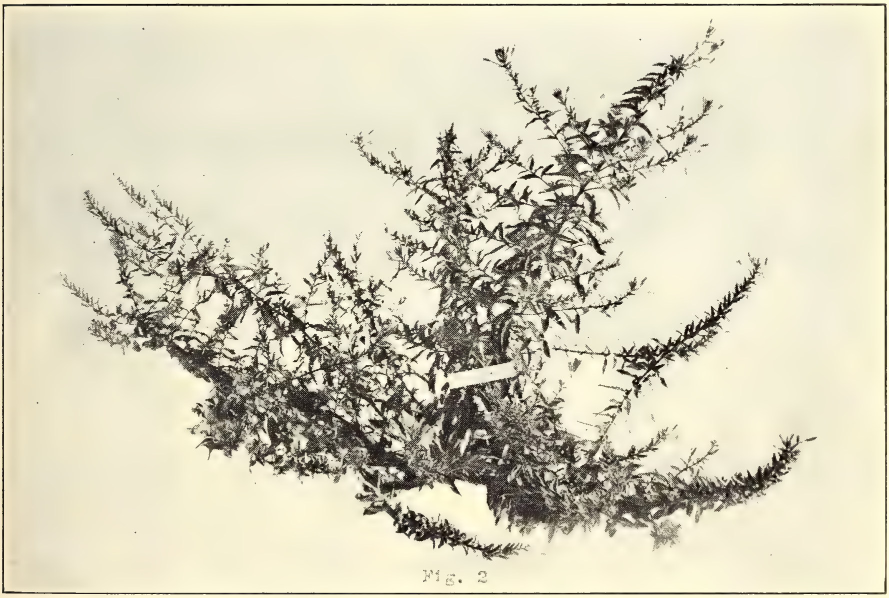
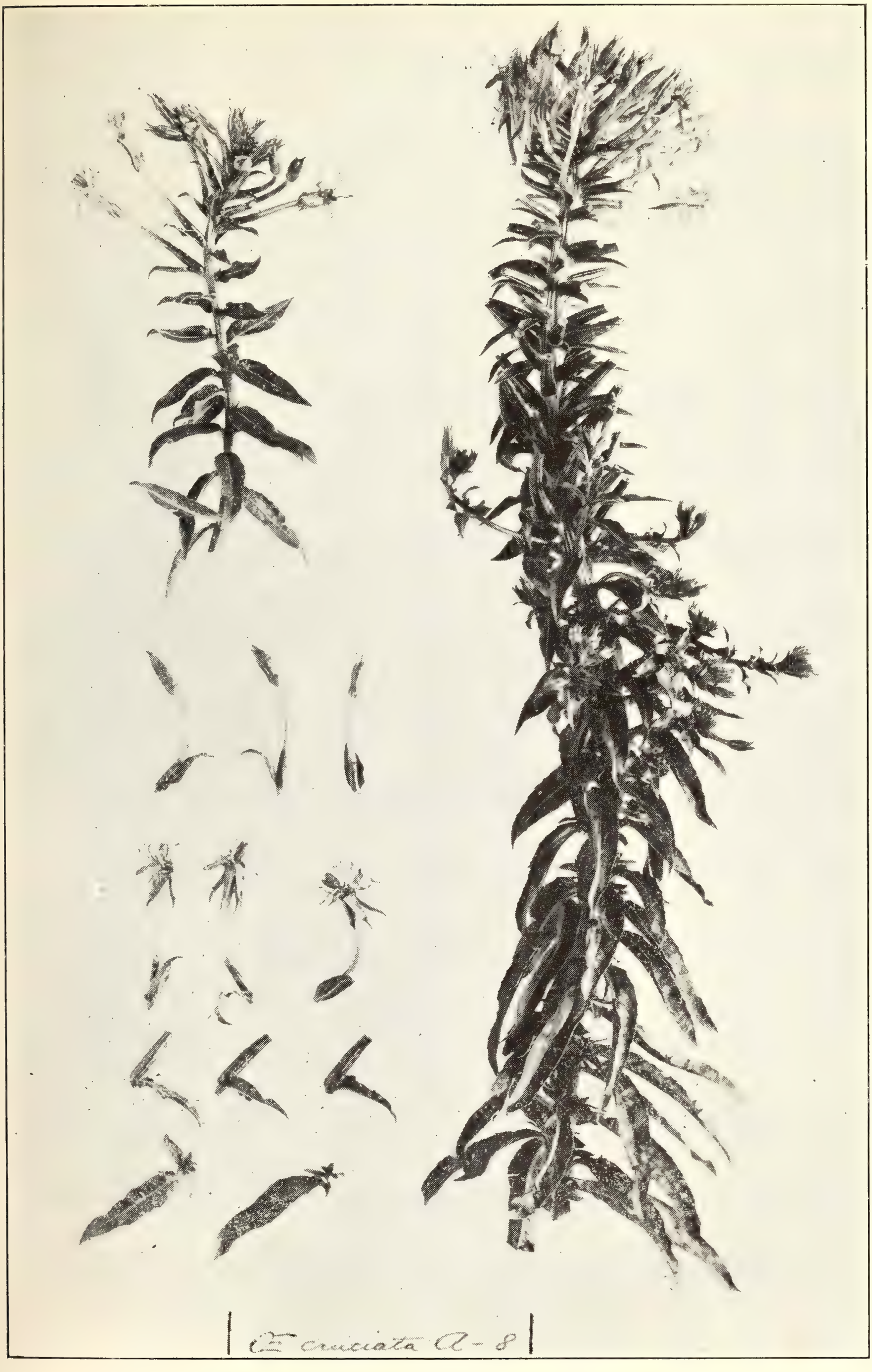
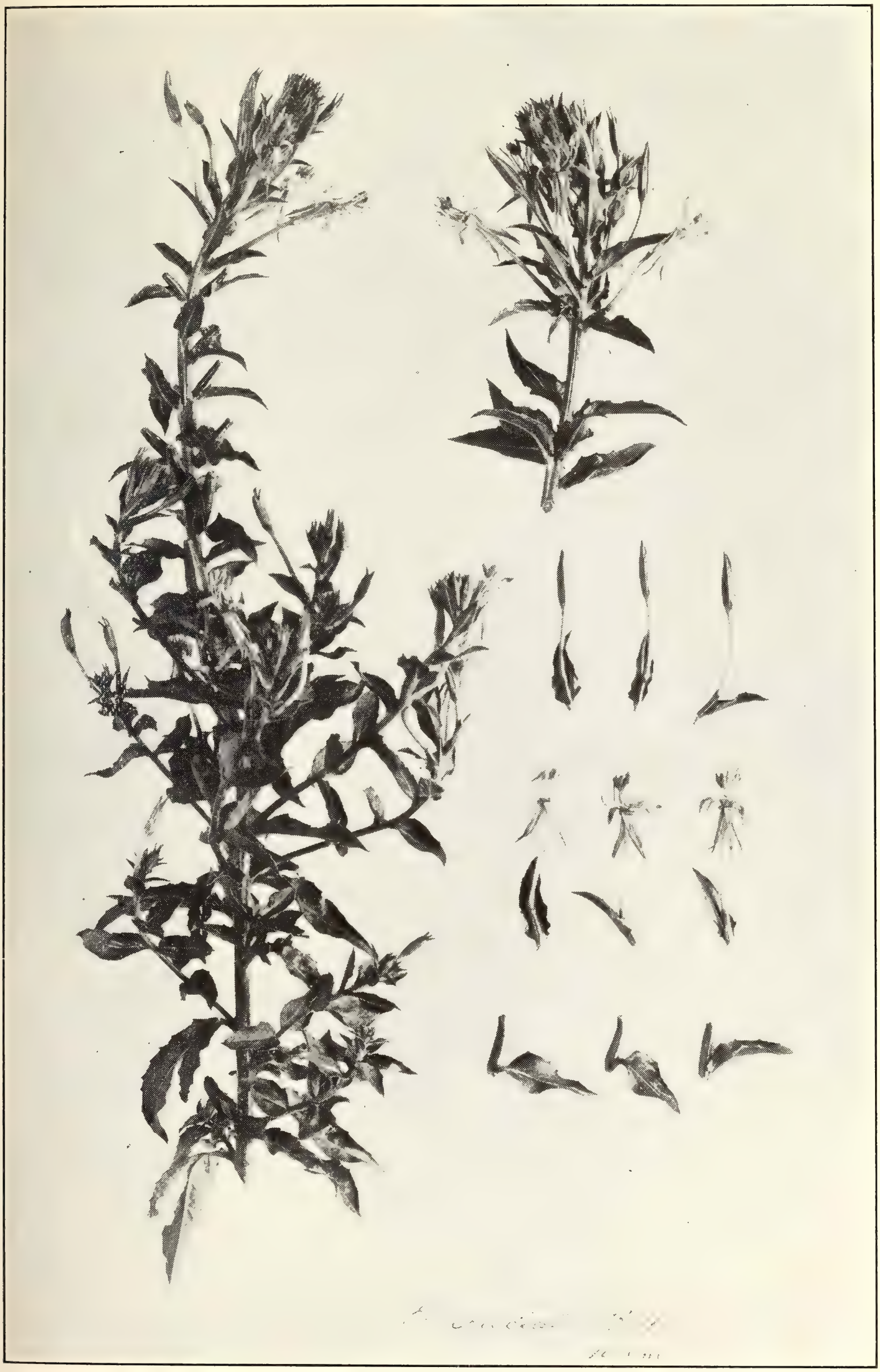


Fig. 2

BARTLETT: CRUCIATE-FLOWERED ŒNOTHERAS.


Œ cruciata A-8

BARTLETT: CRUCIATE-FLOWERED ŒNOTHERAS.



BARTLETT: CRUCIATE-FLOWERED ŒNOTHERAS.

Bethesda, Montgomery County, Maryland. Specimens from garden plants, *Bartlett* 2717 and 3146 (type, in U. S. Nat. Herb. nos. 693733–5.).

Bureau of Plant Industry,
Washington, D. C.

Explanation of Plates XIX–XXI

Plate XIX, Fig. 1. *Oenothera atrovirens* Shull & Bartlett. Flowering plant. (The small label is 10 cm. long.)

Fig. 2. *Oenothera venosa* Shull & Bartlett. Flowering plant. (The small label is 10 cm. long.)

Plate XX. *Oenothera atrovirens* Shull & Bartlett. Inflorescence and details.

Plate XXI. *Oenothera venosa* Shull & Bartlett. Inflorescence and details.

PLUS AND MINUS STRAINS IN THE GENUS GLOMERELLA

C. W. Edgerton

With one figure in text and plates XXII and XXIII.

Certain species of the Mucorineae, or black mold group of fungi, are composed of two distinct strains, either of which is capable of living by itself and of producing asexual spores, but neither is able alone to produce the zygospores or sexual stage. When the two strains are together, however, and the environment is suitable, the sexual spores are produced abundantly. In the formation of the zygospores, one gamete is produced by one strain and the other gamete by the other strain. In this group of fungi, the strains are very similar; in most cases it being impossible to differentiate between them from a macroscopic or microscopic examination. While these two are without doubt sexual strains, their similarity has made it seem advisable to Blakeslee, who has made a study of many of the forms, to use the indefinite terms, plus and minus, in preference to male and female

Outside of the Mucorineae sexual strains in fungi are either very rare or else have not been recognized and it would seem that they are very rare as many unsuccessful attempts have been made to find them. However in the genus Glomerella, which is the perithecial stage of certain species in the form genera, Gloeosporium and Colletotrichum, a condition exists which approaches that found in the Mucorineae.

The ascogenous stages of many of the Gloeosporiums and Colletotrichums have been known for a number of years. In some of the forms, as for example the one causing the bitter rot disease of apples, the perithecial stage is rather common, while in others its development seems to be rare. Yet with all of the forms the perithecia develop very erratically sometimes present in abundance and other times entirely absent. While working on these fungi during the past several years and trying to find out some of the reasons of the erratic development of the perithecia, the writer has constantly had in mind the possibility of sexual strains. Until about three or four years ago, nothing developed which would in the least show that more than one strain was present or was necessary for the development of the perithecial stage. Frequently cultures made from single spores would produce the perithecia, this seeming to prove that the species were homothallic, that is, with both sexual organs on the same strain. But during the past four years, several fungi of this group have been found which develop two strains which behave in some ways similar to the strains of the Mucorineae. There is a fertilization between them followed by the development of an abundance of perithecia where they grow together on culture media (plate I, fig. 2). While the behavior of these strains is not identical in all ways with the strains of the black molds, it seems best for lack of better terms to use here also the indefinite terms, plus and minus.

While working on this problem, cultures from several different host plants have been obtained which have shown the presence of the two different sexual strains. The first one that was studied was obtained in northern Louisiana in August, 1910 from the petiole of a leaf of the cottonwood (*Populus deltoides*). This one has been studied for nearly four years. A preliminary report[1] on this was given at the Washington meeting of the Botanical Society of America in December, 1911. This fungus is a typical Gloeosporium and, from a microscopical examination, it is impossible to tell it from *Gloeosporium fructigenum*, the common form causing the bitter rot disease of apples (*Malus sylvestris*). Other hosts from which the two strains have been obtained are the giant beggar weed (*Desmodium tortuosum*), okra (*Hibiscus esculentus*), and morning glory (*Ipomoea purpurea*). Most of the work discussed in this article was carried on with the two forms from cottonwood and morning glory. The cottonwood fungus has been kept in culture for nearly four years and is still producing perithecia in abundance when the two strains are together, and at no time has it shown any signs of deterioration or running out.

The morning glory fungus is also interesting in another way from the fact that only perithecia develop in cultures. While conidia are usually very abundant in Glomerella cultures, none of these have ever been observed in either strain of the anthracnose collected from the morning glory. This is similar to the ascogenous culture from bean (*Phaseolus vulgaris*) which Shear and Wood[2] have reported. There is no question, however, regarding the identity of this fungus. The perithecia, asci, and ascospores cannot be told in any way from the same structures in other Gloeosporium cultures. Furthermore there were typical conidia along with the perithecia on the morning glory stem from which the cultures were made.

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[1] Edgerton, C. W. Plus and Minus Strains in an Ascomycete. (Abstract) Science, n. s., **35**: 151. 1912.

[2] Shear, C. L., and Wood, Anna K. Studies of Fungous Parasites Belonging to the Genus Glomerella. Bur. Pl. Ind. Bul. **252**: 46–47. 1913.

The presence of two strains was first recognized in the culture from the cottonwood. Plantings in petri dishes often showed a colony development like the one illustrated in Plate I, fig. 1. In the center there was a strict growth, black with perithecia, and generally somewhat stellate in shape. Outside of this there was a floccose growth in which perithecia developed in masses in a manner similar to most described Glomerella cultures. Perithecia on the boundary line between these two different growths were always better developed than the perithecia in the black portion of the colony. The plate looked as if there were two fungi present and that the white one was a more rapid grower and had finally outgrown the other and confined it to the central region; and this was finally proven to be the case. Dilution cultures were made from the two portions of the plate and the two distinct forms of growth were isolated. Transfers made from colonies developing from single spores showed two distinct forms and these are the ones which are designated in this paper as the plus and minus strains. These have been studied on various culture media and under various conditions for several years.

The plus strain grows well on all of the ordinary culture media and in most cases it develops an abundant growth of aerial, floccose mycelium of a white or light gray color (upper right and lower left quarters, Plate II, fig. 1). Perithecia usually develop when grown on a good medium such as bean or oat juice agar and they always form in raised masses or nodules similar to the perithecia of other described species of Glomerella. The asci (fig. 1*b*) and ascospores are always well developed in the perithecia. This strain grows more rapidly than the other and when the two are together, it usually confines the latter to the central region of the colony (Plate I, fig. 1).

The minus strain also grows well on most media but produces scarcely any aerial mycelium. The perithecia are produced in great abundance in and on the surface of the culture medium (upper left and lower right quarters, Plate II, fig. 1), generally single though occasionally in twos or threes. The abundance of perithecia generally gives the culture a black color. On ordinary culture media as potato or bean agar, the perithecia of the forms studied do not mature; they remain small and no asci develop in them. On some special medium such as Clinton's oat juice agar[3] which has been acidified, some of the perithecia come to maturity, but, even on this medium, the asci are usually irregular in shape (fig. 1*a*) and do not seem to have developed properly.

Most of the work in the past on perithecial forms of the genus Glomerella seems to have been done with what is called the plus strain in this article. The description of the genus Glomerella as given by Spaulding and von Schrenk[4] was based on the characters of the plus strain of the fungus that causes the bitter rot disease of apples. The cultures with ascogenous stages used by Miss Stoneman,[5] Clinton,[6] Sheldon,[7] and a few others were apparently all of this strain. Yet there are a few cases in the published literature where it seems almost

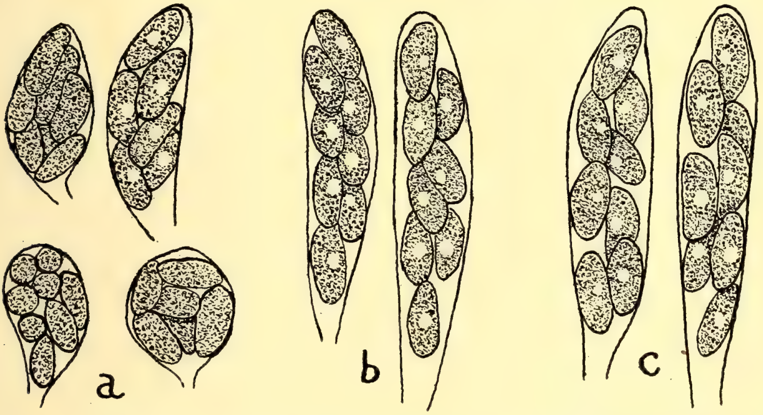

FIG. 1. Asci from the cottonwood Glomerella as formed on oat juice agar: *a*, Asci from the minus strain; *b*, Asci from the plus strain; *c*. Asci from the boundary line between the two strains.

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[3] Clinton, G. P. Oospores of Potato Blight. Conn. Agr. Exp. Sta. Report **1909–1910**: 760–761. 1911.

[4] Von Schrenk, Hermann, and Spaulding, Perley. The Bitter Rot Fungus. Science, n. s., **17**: 750–751. 1903.

[5] Stoneman, Bertha. A Comparative Study of the Development of Some Anthracnoses. Bot. Gaz. **26**: 69–120. 1898.

[6] Clinton, G. P. Apple Rots in Illinois. Ill. Exp. Sta. Bul. **69**. 1902.

[7] Sheldon, J. L. The Ripe Rot or Mummy Disease of Guavas. West Va. Exp. Sta. Bul. **104**. 1906.

certain that the minus strain was also used. In a previous article, the writer[8] described a culture from apple which no doubt belonged to this stage. This culture was explained at the time as a possible mutation but from the observations of the past few years, there seems no doubt but what this was really the minus strain of the bitter rot fungus. Shear[9] also seems to have had both strains of several of the forms on which he has been working though he did not consider them to be sexual strains.

When plantings of the plus and minus strains were made short distances apart on nutrient media in petri dishes and the two developing colonies allowed to grow together, the probability of them being sexual strains became evident. Up to the time the colonies came in contact, the growth of each was similar to that described above. On the boundary line, however, where they were in contact, there developed a black line of perithecia (Plate I, fig. 2). Under suitable temperature and cultural conditions these perithecia from the boundary were mature and shedding their ascospores in four or five days after the strains joined, while the development of the asci in a single strain alone was always much slower. On practically all media tried, especially when acidified, this black line of perithecia developed. On bean agar (Plate I, figs. 2 and 3; Plate II, fig. 1) on which the minus strain produced only immature perithecia, the boundary line was black with perithecia with well-developed asci. On oat juice agar (Plate II, fig. 2) on which the minus strain produced poorly formed asci, the boundary line was a ridge of perithecia sometimes more than a millimeter high with perfectly developed asci. In Plate I, figures 4 and 5, are shown photomicrographs of sections across the boundary line on oat juice agar. On the left side in each case is the minus strain, and on the right the plus strain. In the minus strain may be seen the small immature perithecia. Where the two strains come together, the perithecia are large and well developed and many of them extend up above the culture medium. In Plate I, figure 4, the perithecia on the boundary line are just forming and are still immature while those in figure 5 are well developed and filled with asci. Several hundred of such plate cultures have been made during the past four years and in all cases on a suitable medium this line of perithecia has developed when the plus and minus strains from the same host were used. The four cultures from cottonwood (Plate I, fig. 2), okra (Plate I, fig. 3), morning glory (Plate II, fig. 2), and beggarweed always worked in the same way.

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[8] Edgerton, C. W. The Physiology and Development of Some Anthracnoses. Bot. Gaz. **45**: 395–396. 1908.

[9] Shear, C. L., and Wood, Anna K. Studies of Fungous Parasites Belonging to the Genus Glomerella. Bur. Pl. Ind. Bul. **252**. 1913.

There seemed to be two possible explanations of the formation of the ridge of perithecia on the boundary line between the two strains. It was possible that these two were really different sexual strains of the same fungus or else that the perithecial development was due to some chemical or mechanical stimulus due to the two cultures coming in contact. The latter explanation has been offered by Shear and Wood[10] in their studies on this group. In order to test out this possibility some cultural work was carried on with closely related forms of the genus Glomerella. As is well known, species or races of Gloeosporium and Colletotrichum are very widespread in nature being found on an extremely large number of host plants and many of these are very similar and possibly identical. Cultural work shows the forms to be extremely variable and it is impossible from a morphological study to differentiate between many of them. If the formation of a boundary line of perithecia is due to a chemical or mechanical stimulus, why will not this line develop when one of the strains is grown with other forms? To test this out, the plus and minus strains of the cottonwood and morning glory fungi were grown in the same plate with non-ascogenous cultures of the Gloeosporiums and Colletotrichums isolated from *Malus sylvestris*, *Gossypium hirsutum*, *Capsicum annuum*, *Manihot sp.*, *Melilotus indica*, *Ficus carica*, and *Hibiscus esculentus*. These cultures came in contact with both the plus and minus strains. The plus and minus strains would develop the ridge of perithecia between themselves but in no case would either of the strains develop any perithecia in contact with any of the colonies from the other hosts. Finally to test this out further, another culture was isolated from leaves of *Populus deltoides* in 1912. This proved to be a non-ascogenous culture. This was grown in the same plate with the ascogenous plus and minus strains from the same host but the line of perithecia would not develop between it and either of the strains.

Having been unable to develop a boundary line of perithecia between either the plus or minus strain and any of the other non-ascogenous anthracnose cultures, an attempt was made to produce this between these strains and ascogenous cultures from other hosts. Plantings were made in the same plate of both of the strains from the cottonwood and also both of the strains from the morning glory so that either strain would come in contact with both of the opposite strains. As the colonies developed, the plus strain of each host came in contact with both of the minus strains. Perithecial ridges developed between the plus and minus strains of the cottonwood fungus and between the plus and minus strains of the morning glory fungus, but no sign of a ridge formed between the plus strain of the cottonwood fungus and the minus strain of the morning glory fungus or between the minus strain of the cottonwood fungus and the plus strain of the morning glory fungus. One of these plates is shown in Plate II, figure 1. The prominent perithecial ridges are between the plus and minus strains of the same host.

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[10] Shear, C. L., and Wood, Anna K. Studies of Fungous Parasites belonging to the Genus Glomerella. Bur. Pl. Ind. Bul. **252**: 74. 1913.

These experiments seemed to cast doubt on the possibility of this perithecial development being due to a chemical or mechanical stimulus. It would seem, if this were the cause, that some of these very closely related forms would also have provided this stimulus. These experiments strengthened the theory that the perithecial development was really due to a fertilization between two sexual strains.

To prove that there was really a fertilization between the strains was a rather difficult proposition. The mycelial development is so profuse in the plates that it is impossible to tell by examination whether both strains enter into the formation of the perithecium or not. In order to get some data on this point, an attempt was made to culture the ascospores that developed in a single perithecium on the boundary line. The ascospores of a Glomerella are shed very quickly after they form and they ooze out of the perithecium and remain in a little droplet at the orifice (Plate I, fig. 6). Dilution cultures were made from these little droplets of spores that had oozed out of single perithecia. If there was a cross fertilization, it would be natural to suppose that the spores developing in a single perithecium would develop into two strains while if there were no crossing and the ridge of perithecia on the boundary line was but due to some chemical stimulus, all of the ascospores would develop into but a single strain in the same manner as all of the ascospores from a single perithecium from a single strain do. Plates from nineteen different perithecia were made and of these seventeen showed colonies of both strains. In the other two only the minus strain seemed to be present though the colonies were so numerous in these that they could not be told with certainty. There was some objection to this method, however, and a slight uncertainty because no matter how careful one might be in transferring from a single droplet of ascospores, there was a slight chance of other loose ascospores being present.

In order to eliminate all of the uncertainty in regard to the strains present in a single perithecium, it was decided to isolate single asci from the boundary line of perithecia and let the different ascospores in them germinate and see if the different ones from a single ascus would develop one or both strains. The isolation of an ascus from a culture of this genus is a difficult operation on account of the fact that the spores are shed as soon as the ascus is mature. In examining a mount made from perithecia, there will be a few scattering asci with mature ascospores present, a great many immature asci generally held in clumps though often loose, and a multitude of loose ascospores. It was found impossible to plate these out dilute enough and then find an ascus in the plate. The only way that seemed possible to isolate the ascus was by some method to pick it up from the mass of spores and transfer it to a marked place in a plate. After many unsuccessful attempts this was found to be possible by attaching a very fine capillary tube which was sealed at the large end to the substage mechanism of the microscope.[11] The capillary tube was firmly held and could be moved in any direction by the various screws which regulate the position of the condenser of the microscope. By means of this capillary tube, the ascus was picked up and then transferred to a sterilized cover slip. The small drop of water on the cover slip was then examined and if only the ascus was found to be present, the cover slip was pushed down into a plate of sterile agar. If other spores were transferred with the ascus, the latter was again picked up and transferred to another cover slip in the same manner or else it was discarded. This was a slow process but it insured the isolation of the ascus. After the ascus was placed in the culture medium, its position was marked on the bottom of the plate with a blue pencil. It was then watched while the spores in it were germinating to make absolutely sure that no other spores were present. The ascospores would germinate in a few hours and finally they would develop into a single colony. The presence of one or both strains could usually be told by looking at the colony; but, to make absolutely sure, several times they were reisolated by dilution cultures.

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[11] Edgerton, C. W. A Method of Picking up Single Spores. Phytopathology 4: 115–117. 1914.

Over forty different asci were transferred, but the ascospores in some failed to germinate and some of the plates were so badly contaminated with bacteria that they had to be discarded. From twenty one colonies that did develop from single asci, twelve contained both the plus and minus strains and nine contained only the minus strain. In Plate II, figure 2, is shown a petri dish in which all of the colonies developed from spores that formed in one of the colonies from a single ascus. Both plus and minus strains are present. This shows that not only were there both strains present in a single perithecium but that they were also present in a single ascus, and this would seem to prove conclusively that there was an actual fertilization between the two strains on the boundary line where they come together in a plate, that one strain furnished the antheridia and the other the oogonia.

All the asci did not produce both the strains but this was hardly to be expected. The culture medium used was oat juice agar, and, as has been noted, some of the perithecia of the minus strain mature on this medium and the asci in these as a rule do not break up as readily as the well developed asci that normally form in the boundary line, and it is possible that some of these might have been transferred. The perithecia of the minus strain develop abundantly all over the surface of the colony and it would be possible to get some of them in a mount made from the boundary. The plus strain only develops in nodules and the chances would not be good for transferring any of these to a mount, and as can be seen there were none of the asci that developed the plus strain alone. Furthermore it is possible that some of the asci that were transferred were not mature. Immature asci are always more abundant in a mount than mature ones on account of the disintegration of the latter at maturity. Whether the immature ascus as a whole would germinate has not been determined, but if it would, the resulting colony would probably be of the minus strain as this is probably the strain that produces the oogonia. And then it is possible that some of the ascospores in an ascus would get started before the others and prevent the latter from developing to any extent. However, the presence of both strains in any of the asci demonstrates the fact that there is really a fertilization between the colonies and that the plus and minus strains should be classified as sexual strains.

The process of fertilization has not been studied to any great extent as yet and it is impossible to explain with certainty the phenomena which have been described in this article. However, a theory that would seem to explain the facts described may be of interest. The minus strain produces an abundance of perithecia and it would seem that this is the strain that produces the oogonia and might be designated as the female strain. As most of the perithecia remain immature or at most but poorly developed, it would seem as if the antheridia in this strain were mostly lacking or poorly developed so that the stimulus following the fertilization is not sufficient to bring the perithecia to maturity. With special stimulating media, the antheridia are possibly better developed or else the stimulating effect of the medium added to that of the fertilization is sufficient to bring some of the asci to maturity. In the plus strain, it is probable that the antheridia are produced very abundantly while the oogonia are produced only sparingly, and where the latter are produced the perithecia form in masses. If the two strains are brought together on the same plate, fertilization takes place and a profuse development of perithecia follows.

It would seem that we have conditions in this group somewhat intermediate between conditions usually found in the Mucorineae. In such fungi as *Rhizopus nigricans*, only one set of the sexual organs is produced on a single strain and the presence of both strains is necessary for the production of the zygospores; while in species like *Sporodinia grandis*, both sexual organs are produced on the same strain. In the Glomerella fungi we have forms in which a fertilization between strains is not necessary for, but stimulates the production of the sexual stage.

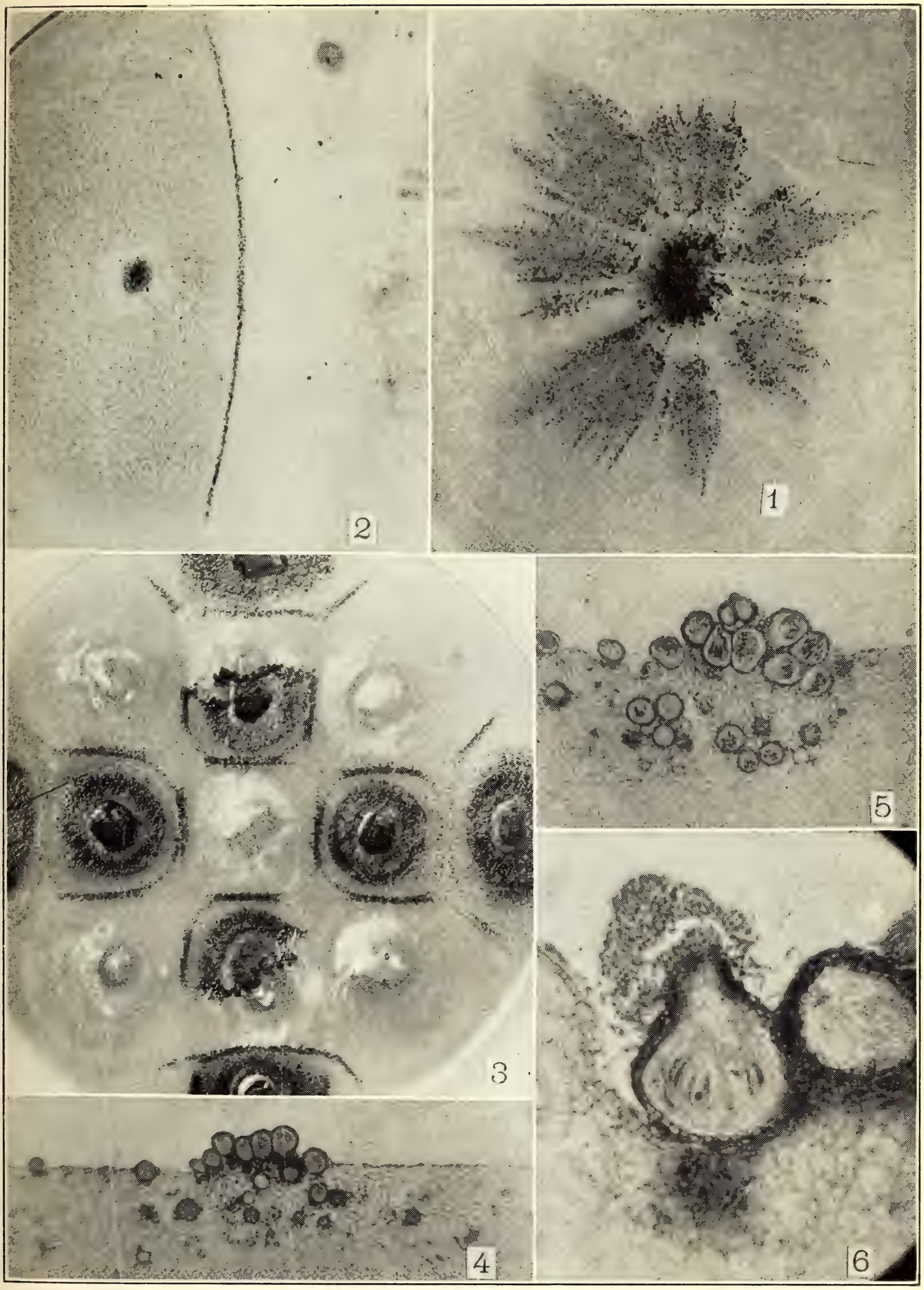
Whether other forms of the ascomycetes may be found in which a fertilization between different strains stimulates the development of the fruit body and ascospores is a question. The Glomerella forms show so much variability that it is possible that we have in the cultures that have been described merely isolated variations that may not be common in the ascomycetes as a group or even within the genus Glomerella itself. Yet the fact that two sexual strains may sometimes be present adds another factor to be considered in all work dealing with the development of ascogenous stages.

Louisiana Agricultural Experiment Station,
Baton Rouge, La.

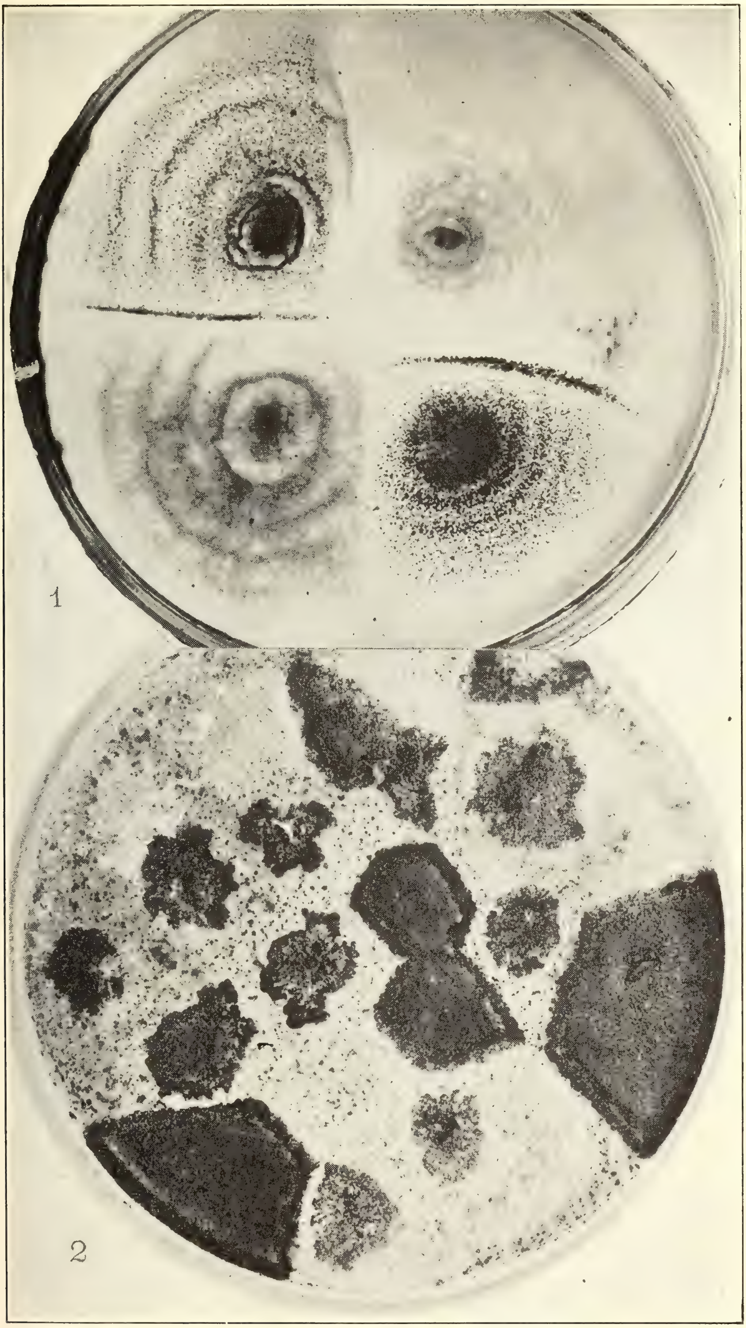
Explanation of Plates XXII and XXIII

Plate XXII. Plus and minus strains of Glomerella. 1. Plus and minus strains of the cottonwood Glomerella in a single colony, showing the minus strain with the numerous perithecia confined in the central portion of the colony with the white plus strain on the outside. 2. Plus (right) and minus (left) strains of the cottonwood fungus with the line of perithecia between them. 3. Plus and minus strains of the Glomerella from okra. 4. Section across boundary line between plus and minus strains; perithecia just forming. 5. Section across boundary line between plus and minus strains; perithecia mature. 6. Perithecium from the boundary line between the plus and minus strains showing the ascospores in a droplet at the orifice.

Plate XXIII. Plus and minus strains of Glomerella. 1. Plus and minus strains of the Glomerellas from cottonwood (left) and morning glory (right) showing the boundary line of perithecia between the two strains from the same host but not between the two strains from different hosts. 2. Plus and minus colonies of the morning glory Glomerella on oat juice agar, isolated from a colony that developed from a single ascus from the boundary line between the two strains.



EDGERTON: PLUS AND MINUS STRAINS IN GLOMERELLA.



Edgerton: Plus and minus strains in Glomerella.

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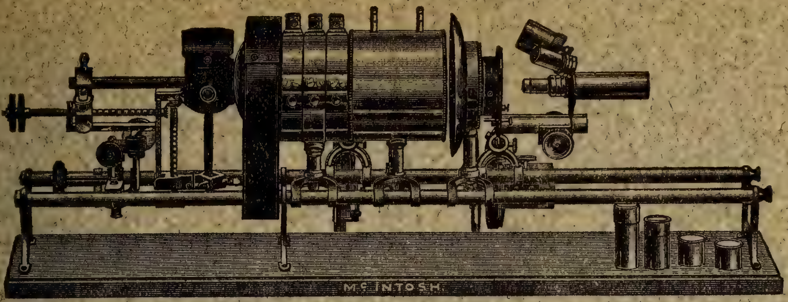

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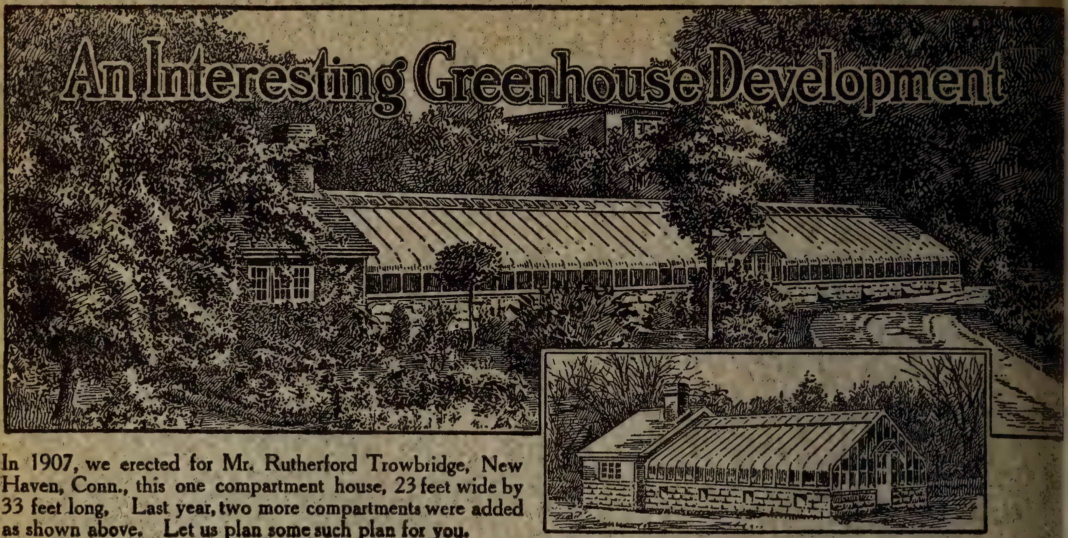

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THE HARMFUL ACTION OF DISTILLED WATER[1]

Rodney H. True

Historical Introduction

The use of distilled water in experimental biological work introduced a group of problems some of which still demand the attention of the investigator. The use of this medium was begun and has continued because of the desire of the experimenter to test the effect of pure water, not only for the purpose of furnishing a check on the action of aqueous solutions of various substances under investigation, but also in order to meet the necessity of providing the pure substance for the study of the physiological action of water itself.

It was early recognized that for chemical purposes distillation furnished a convenient means of ridding water of a great part of its impurities, and for many years distilled water was accepted as being pure water, and its effects were accepted as those characteristic of pure water.

Harmful Action Recognized.—The essentially harmful action of distilled water was suspected, however, at a relatively early date by the plant physiologists, Sachs (1), Knop (2), Boehm (3), Dehérain (4) and others. It was the general opinion that rain water, river water and water from other natural sources exerted a more favorable action on their experimental plants than distilled water and the use of natural waters was frequently resorted to in order to secure what was regarded as normal plant behavior. This harmful action was thought to be due chiefly to the lack of nutrients and less often to actively injurious qualities.

At about the same time the deleterious effect of distilled water on

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[1] Published by permission of the Secretary of Agriculture.

[The Journal for May (1: 203–254) was issued 20 June 1914.]

the structure or functions of animals was noted by Kölliker (5) working on the irritability of nerves, by Nasse (6) dealing with the supporting power of various solutions for frog's muscle, and a little later by Sydney Ringer (7) working on the survival of fishes in different media.

Harmful Impurities Sought.—The existence of harmful properties in distilled water being recognized, many investigators working along several lines of biological and chemical study have endeavored to ascertain the reason for the results observed.

As would be expected, the search for poisonous substances in the water has been thoroughly prosecuted and many investigators have believed the problem solved by results obtained along this line.

In 1891, Loew (8) called attention to the work done on distilled water by Nägeli (9) in an investigation probably begun in the early eighties, but unpublished until 1893 after his death. In this paper, he showed that distilled water from metal stills was contaminated by compounds of the metals coming in contact with the water. These results confirmed by Loew (8), Locke (10), Ringer (11), Dehérain & Demoussy (12), Bokorny (13) and others working in diverse fields of biological research, seemed greatly to simplify the distilled water question. Water distilled from glass was regarded as a well understood and safe medium for physiological work. This comfortable conclusion, however, was not allowed to stand unchallenged.

More recently a number of investigators have asserted that water distilled from glass even with the usual precautions is harmful, through the action of impurities present. Lyon (14) and later Livingston (15) and his associates in the Bureau of Soils of the U. S. Department of Agriculture convinced themselves that distilled water thus prepared contained both volatile and non-volatile unknown poisons. Lyon suggested that dissolved ammonia might be the harmful agent.

Harmful Properties of Pure Water.—Early in the history of the problem it was noted by Knop (16), Zöbl (17) and others among the plant physiologists and by Paul Bert (18), Plateau (19, 20) and Sydney Ringer (7) among the animal physiologists that organisms lose salts when brought into solutions relatively lacking in these constituents and the harmful effects due to this extraction of materials was pointed out. Detailed information concerning the materials extracted was worked out by Knop (16) and Zöbl (17), while Ringer (7) hazarded some well considered surmises. This study was prolonged by Ringer (21–24) and his associates through a number of succeeding years by the use of a variety of methods and of experimental subjects.

The development of the more refined methods of physical chemistry brought forward evidence which when applied to distilled water as a beverage and as a therapeutic agent taken in by way of the stomach, aroused much discussion. Koeppe (25) maintained that distilled water was a poison when drunk in quantity and exerted its harmful action through its powerful osmotic properties. By means of these, it was contended that the cells of the stomach membranes were injured and salts were extracted from the organism with results serious to health. He was supported by Oldham (26) and others. A spirited defense by Winckler (27), Kobert (28) and others followed; the long and favorable experience of practitioners who had found distilled water a harmless and valuable remedy was pointed out. It is clear from reading the literature that while Koeppe was speaking of water considered pure from the standpoint of the physical chemist, his opponents regarded rain water, commercial distilled water and similar somewhat contaminated waters as the physiological equivalent of Koeppe's medium. The fallacy of this assumption was not realized by them.

The more recent studies of Jacques Loeb (29, 30), A. W. Peters (31) and others have been built essentially on the foundation laid by Ringer and other predecessors. It is agreed that the extraction of substances from the animals used as test objects is accomplished by distilled water, the line of argument advanced by Sidney Ringer and Koeppe being accepted in so far as the general method of working injury is concerned. The advance over their views is found to consist chiefly in the deeper penetration into the cellular operations involved. While Ringer and Koeppe were content to say that salts essential to the maintenance of the integrity of the structure and function of the organism were extracted by distilled water, Loeb seeks to relate the salts extracted to certain necessary ion-proteid compounds.

Experimental Evidence

The results here given were worked out in the winter and spring of 1905, and in part in the winter of 1906. The preparation of the distilled waters and of the few solutions studied in this connection was kindly done at the writer's request by Dr. Lyman J. Briggs, at that time soil physicist of the Bureau of Soils, U. S. Department of Agriculture. The help of Dr. G. F. Klugh, at that time the writer's assistant, was obtained during the winter of 1906. The results and conclusions here given are regarded as a preliminary contribution to the solution of the problem.

Materials and Methods.—The distilled waters were prepared in Dr. Briggs's laboratory, under his supervision. As test objects, the radicles of *Lupinus albus*, the white lupine, were chosen. This genus had been shown by Frank (32) to be very sensitive to distilled water injury, a fact rendering it an especially favorable subject for study in this connection. The radicles were suspended in beakers containing the culture solutions, glass hooks being used so to hold the seedlings as to suspend them with their roots in the solutions. These hooks were supported by a cork sheet covering the whole beaker through which they were thrust. The beakers used were made of glass of the so-called "nonsol" formula and had a capacity of 300 c.c. About 150 c.c. of the solutions were used in each culture of four roots. These beakers had never been used, and were prepared by being thoroughly washed and steamed out. In order to get numerical data for comparison, the growth rate of the roots was adopted as a criterion, and the standard period of time between measurements was twenty-four hours. A fine line of India ink was fixed at a distance of 15 mm. from the tip, and the change in length of this region included the entire growth in length of the radicle. The seeds were germinated in moist, chopped sphagnum carefully washed out, and the seedlings before setting into the culture medium were carefully rinsed in a duplicate portion of the same.

As a check medium, tap water was chosen. This is drawn from the Potomac River, some miles above the city, filtered on a large scale, and delivered to the city through the usual appliances for distribution. Analyses of filtered Potomac water covering the entire period of our experiments are not available, but an average of the analyses made by Outwater (33) covering twelve months, between May, 1904, and August, 1905, is as follows:

	Parts per million
SiO_2	5.15
F_2O_3, Al_2O_3	4.63
Ca	30.94
Mg	4.62
Na	3.20
K	0.62
Cl	5.02
SO_4	8.68
CO_3	2.03
HCO_3	97.77

During this period, the Ca content varied between 10.37 parts (May, 1904) and 54.41 parts per million (Dec., 1904); the Mg content between 1.87 parts (May, 1904) and 8.29 parts (Dec., 1904); the K content between a "trace" during 9 months of the period and 5 parts per million in June, 1904.

The beakers containing the cultures, when not under observation, were shut in a dark laboratory cupboard, where the temperature was maintained between 20° and 22.5° C.

Owing to the limits set on the development of lupine roots by distilled water, extended experiments could not be carried out. It is clear, therefore, that the behavior of plants during long periods should be investigated with peas, maize, or other species which are less sensitive to the influences exerted by distilled water.

Effect of Using Condensers Made of Different Materials

It was thought desirable, first of all, to establish the relation between the growth rate in Potomac water and in distilled waters made by using condensers of various materials. Accordingly, water was distilled from glass, the vapors being condensed in copper, tin, platinum and glass condensers, respectively. The resulting waters were tested in two different experiments, the following summary presenting the average of the two series of plants:

Table I

Waters Condensed in Different Materials

Temp. 20° to 22.5° C.

Source of water	Growth 1st 24 hrs., mm.	Growth 2d 24 hrs., mm.
Copper Condenser	14.5	6.2
Tin Condenser	15.7	6.5
Platinum Condenser	18.2	6.7
Glass	16.2	6.5
Potomac water	23.5	27.5

From this experiment it is clear that all distilled waters were distinctly harmful in their action during the first 24-hour period, the advantage being with the platinum condenser. According to Nägeli (9), Loew (8) and others, glass apparatus is supposed to deliver physiologically safe distilled water. It is of interest in this connection to note that Copeland and Kahlenberg (34) found that lupine roots grown in containers in which considerable areas of pure platinum metal were allowed to remain throughout the duration of the experiment showed a greater growth after 11 days than did the roots in the control culture of distilled water. Copper is here the most harmful. During the second 24-hour period, no clear advantage seems to lie with any kind of material. The cause of damage present in all of these distilled waters continues to operate throughout the period of the experiment.

In view of the harmful action of the water distilled from glass, it seems to follow that metal carried over from the apparatus can hardly be invoked as the fundamental source of trouble.

Effect of Distillation Process Compared with Freezing Out

It being clear that all waters purified by distillation were harmful compared with tap water, the question next arose as to whether the process of distillation itself, in the various kinds of apparatus used, was the source of trouble, or whether water purified by other means would act like distilled water. Accordingly, clear, natural ice was melted and refrozen three times in vessels containing no metal, the water not being allowed to warm to room temperature until after the last recrystallization. Comparison cultures of distilled water condensed in copper and platinum and a check in Potomac water were set up.

Table II

Waters Purified by Distillation and by Recrystallization

Method of purification	Growth 1st 24 hrs., mm.	Growth 2d 24 hrs., mm.
Recrystallization	13.0	5.0
Copper condensed	15.0	7.5
Platinum condensed	15.0	6.4
Potomac water	20.7	17.0

It appears from these results that water purified by repeated recrystallization is fully as harmful to lupine roots as that purified by distillation. This result suggests two explanations of the cause of trouble—either the water is harmful because of traces of toxic substances left in it by both the recrystallization and the distillation processes, or it is more harmful than in its original state because of the loss of substances there present.

Leaching Action of Distilled Water.—It has long been known that seeds, when soaked for long periods in water, give up salts to the water in quantities sufficient to permit analysis by the ordinary methods. This work has been repeated and extended by André (35, 36) to include potato tubers as well as seeds, and it seems clear that all of these structures give off salts to relatively pure water. Sachs (37) at an early date showed that this conclusion should be extended to include leaves also.

Physiologists working on animals have made it appear probable that distilled water is able to withdraw salts from animal tissues also, and have advanced the view that harmful results seen to follow a prolonged stay in distilled water are due to the leaching of salts required for the maintenance of normal conditions in the tissues.

The responsibility of distilled water for certain untoward catarrhal conditions of the stomach supposed by some to be associated with the prolonged use of distilled water has been much debated. It is stated by Findlay (38) and others that when copiously used, distilled water leaches the salts and perhaps other materials from the stomach linings with resulting injury to the membranes. On the other hand, distilled water has found active defendants who contend that it is harmless when thus used.

Since it seemed doubtful in the course of the above investigations that the harmful results produced by distilled water on lupine seedlings could be laid at the door of impurities dissolved in the water, attention was given to the alternative possibility that the water was harmful because of its lack of dissolved substances. Again, the electrical conductivity of the water was used as a means of ascertaining the degree of purity. Of course, it need hardly be pointed out that this test is at best but an imperfect one, since non-electrolytes might be present in the solution and remain undetected. Indeed Knop (2) has shown that distilled water extracts some organic as well as inorganic constituents from seeds of peas and corn. Since, however, there was no better method available, it was accepted as affording valuable data.

Accordingly, a series of cultures was set up which were designed to test the supposition that salts might be leached from the roots. It seemed likely that if leaching takes place, a greater amount of salts would be withdrawn into an outside volume of 150 c.c. of distilled water from several roots than from a less number.

TABLE V

LEACHING ACTION OF DISTILLED WATER IN 24 HOURS

Medium	Number of roots	Growth in 24 hrs., mm.	Electrical conductivity × 10^{-4}
Distilled water.........	10	....	2.32
Distilled water.........	4	11.2	1.43
Distilled water.........	0	....	0.82

This result shows that a volume of distilled water to which four lupine roots are exposed for 24 hours gains markedly in conductivity in comparison with a like volume of the same medium standing under like conditions, but containing no roots. The increase in conductivity per root is about 0.151 × 10^{-4} in twenty-four hours. When instead of four roots ten are present in the medium, the total fall in resistance is distinctly greater, indicating the corresponding leaching action of the distilled water. It is to be noted also that the rate of increase in conductivity per root is about the same as that seen in the series of four individuals, viz., about 0.155 × 10^{-4}.

It was next desired to test this same action over longer periods of time, in order to ascertain whether the extraction process was continued.

Cultures containing 150 c.c. were set up, containing ten roots, four roots, and no roots, in three series, to run for 48 hours and 72 hours, respectively.

TABLE VI

LEACHING ACTION OF DISTILLED WATER DURING 48- AND 72-HOUR PERIODS

Medium	Duration of test, hrs.	No. of roots in culture	Growth rate			Electrical conductivity at end of period × 10^{-4}
			1st 24 hrs., mm.	2d 24 hrs., mm.	3d 24 hrs., mm.	
Distilled water....	48	10	....	....	....	4.35
	72	10	....	....	....	4.76
Distilled water....	48	4	9.5	2.5	....	1.87
	72	4	9.5	3.0	2.5	2.06
Distilled water....	48	0	....	....	....	0.80
	72	0	....	....	....	0.75

This table shows that both in the cultures containing 4 roots and 10 roots, respectively, the leaching of ions into distilled water continues, but at a slightly diminished rate in comparison with that seen in the first 24-hour period, the total leaching for the 48-hour period being relatively greater than for the 72-hour period.

In order to bring out the relative rate of extraction more clearly, the following table is compiled from the foregoing data. It shows the rate of leaching as gain in conductivity expressed in units per root per day of 24 hours.

TABLE VII

RATES FOR LEACHING PER ROOT PER DAY

Time of experiment	Four roots	o ts
24 hours..............	0.151×10^{-4}	0.155×10^{-4}
48 hours..............	0.135×10^{-4}	0.177×10^{-4}
72 hours..............	0.110×10^{-4}	0.134×10^{-4}

This comparison shows that in the experiment with 4 roots the leaching continues through the longest period tested, 72 hours, but at a clearly decreasing rate. The regularity seen here is not found in the experiment with 10 roots. It is interesting to note in this connection that the conductivity of the check decreases during the longer periods, a point to which attention will be recalled later. It is important in this connection to note that the growth rate of the culture containing 4 roots falls off in a manner suggesting a parallel with the decrease in resistance. As the distilled water progressively extracts electrolytes, and perhaps other substances, from the plant the growth rate diminishes and almost ceases. In view of the fact demonstrated in earlier studies that the primary radicle of the white lupine seems to be unable to recover in distilled water, seedlings were not exposed for a longer period.

The results seen above seem to indicate that the leaching process begins actively when the roots enter the solution, and during the 72-hour period in which the roots remain there continues at a somewhat decreasing rate. It was thought desirable to follow somewhat further the course of the changes in conductivity and to try to ascertain whether with the accumulation of the leached materials the extraction of the plants continued. Accordingly cultures were arranged in which six series of plants were used: (1) In 150 c.c. of distilled water, 4 plants; (2) in the same volume, 10 plants; (3) in the like volume, no plants; (4) in the like volume of Potomac water, 4 plants; (5) in the same volume of Potomac water, 10 plants, and (6) in the same volume of Potomac water, no plants. Since it was desired to test the physiological properties of these media as affected by the gain of solutes from the leached roots or by the loss of dissolved material through the absorption of such by the roots, each culture container received a new set of seedlings at the end of each three days. This period was chosen since, judging by the growth rate and appearance, it was deemed as long a period as lupine roots could be relied upon to survive the action of distilled water. Post mortem leaching was expressly avoided since it would greatly complicate the situation. Conductivity readings were made at the end of each 72-hour interval. In the case of the check cultures of distilled water and of Potomac water in which no plants were placed, the first readings given represent the conductivity of these latter waters at the beginning of the experiment.

The general features of the result stand out clearly. As the distilled water continues to receive the successive lots of roots the leaching of these structures continues throughout the experiment, as is shown by the steadily increasing electrical conductivity.

The conductivity of the culture containing 10 roots rises more rapidly than that containing 4 roots. At the close of the experimental period of 21 days the conductivities representing the total result of the changes of each culture were as follows: The check containing no roots had risen from a conductivity of 0.87×10^{-4} to 1.61×10^{-4}, or in terms of the equivalent concentrations of KCl from $0.87M/45{,}500$ to $1.61M/45{,}500$, an increase of $0.74M/45{,}500$ KCl. The culture containing 4 roots showed an increase in conductivity from 0.87×10^{-4} seen in the water before receiving the plants to 7.4×10^{-4} corresponding to a change from $0.87M/45{,}500$ to $7.4M/45{,}500$, an increase of $6.53M/45.500$ KCl. Since during this interval the check attained a conductivity of $1.61M/45{,}500$ from sources apart from the plants, the roots had contributed the equivalent of $5.8M/45{,}500$ KCl to the solution, or about $1.45M/45{,}500$ KCl per root. The culture containing 10 roots showed a change in conductivity from $0.87M/45{,}500$ to $12.9M/45{,}500$, a total gain due to the roots of $11.3M/45{,}500$ KCl, or about $1.13M/45{,}500$ per root. It will be noted that the leaching process seems to have been somewhat less active in the culture containing the larger number of roots than in that containing the smaller number.

The plants in the Potomac water check series absorbed electrolytes throughout the entire period of the experiment, and before the close of the interval of observation the river water culture containing 10 roots contained a less quantity of electrolytes than either of the distilled water cultures at the same time and the Potomac culture containing 4 roots contained approximately the same quantity of electro-

TABLE VIII

LEACHING ACTION OF DISTILLED WATER DURING LONGER PERIODS

Medium	Number of plants used	Total growth 72 hours, mm.	Electrical conductivity $\times 10^{-4}$
Dist. water	4 (1st set)	13.0	2.08
Dist. water	10 (1st set)	12.0	3.85
Dist. water	0	....	0.87[2]
Potomac water	4 (1st set)	25.0	30.3
Potomac water	10 (1st set)	26.0	24.1
Potomac water	0	....	37.0[2]
Dist. water	4 (2d set)	9.0	2.94
Dist. water	10 (2d set)	11.5	6.94
Dist. water	0	....	0.86
Potomac water	4 (2d set)	26.0	23.8
Potomac water	10 (2d set)	21.5	15.9
Potomac water	0	....	33.3
KCl. *M*/1,000	....	....	40.0 ?
Dist. water	4 (3d set)	9.5	3.85
Dist. water	10 (3d set)	12.0	8.00
Dist. water	0	....	0.97
Potomac water	4 (3d set)	23.0	22.2
Potomac water	10 (3d set)	21.0	12.2
Potomac water	0	....	34.5
KCl. *M*/1,000	....	....	45.0
Dist. water	4 (4th set)	10.5	4.76
Dist. water	10 (4th set)	12.5	9.05
Dist. water	0	....	1.12
Potomac water	4 (4th set)	25.5	19.6
Potomac water	10 (4th set)	20.5	8.7
Potomac water	0	....	35.7
KCl. *M*/1,000	....	....	45.4
Dist. water	4 (5th set)	11.7	5.46
Dist. water	10 (5th set)	13.5	9.80
Dist. water	0	....	1.22
Potomac water	4 (5th set)	26.5	17.5
Potomac water	10 (5th set)	24.4	9.5
Potomac water	0	....	37.0
KCl. *M*/1,000	....	....	45.6
Dist. water	4 (6th set)	10.0	6.41
Dist. water	10 (6th set)	11.0	11.4
Dist. water	0	....	1.37
Potomac water	4 (6th set)	21.7	15.0
Potomac water	10 (6th set)	17.7	4.31
Potomac water	0	....	37.0
KCl. *M*/1,000	....	....	45.6
Dist. water	4 (7th set)	18.5	7.4
Dist. water	10 (7th set)	17.0	12.9
Dist. water	0	....	1.61
Potomac water	4 (7th set)	46.0	13.5
Potomac water	10 (7th set)	27.0	4.65
Potomac water	0	....	38.4
KCl. *M*/1,000	....	....	45.4

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[2] Determinations made at the beginning of the experiment, representing original conductivity of these waters.

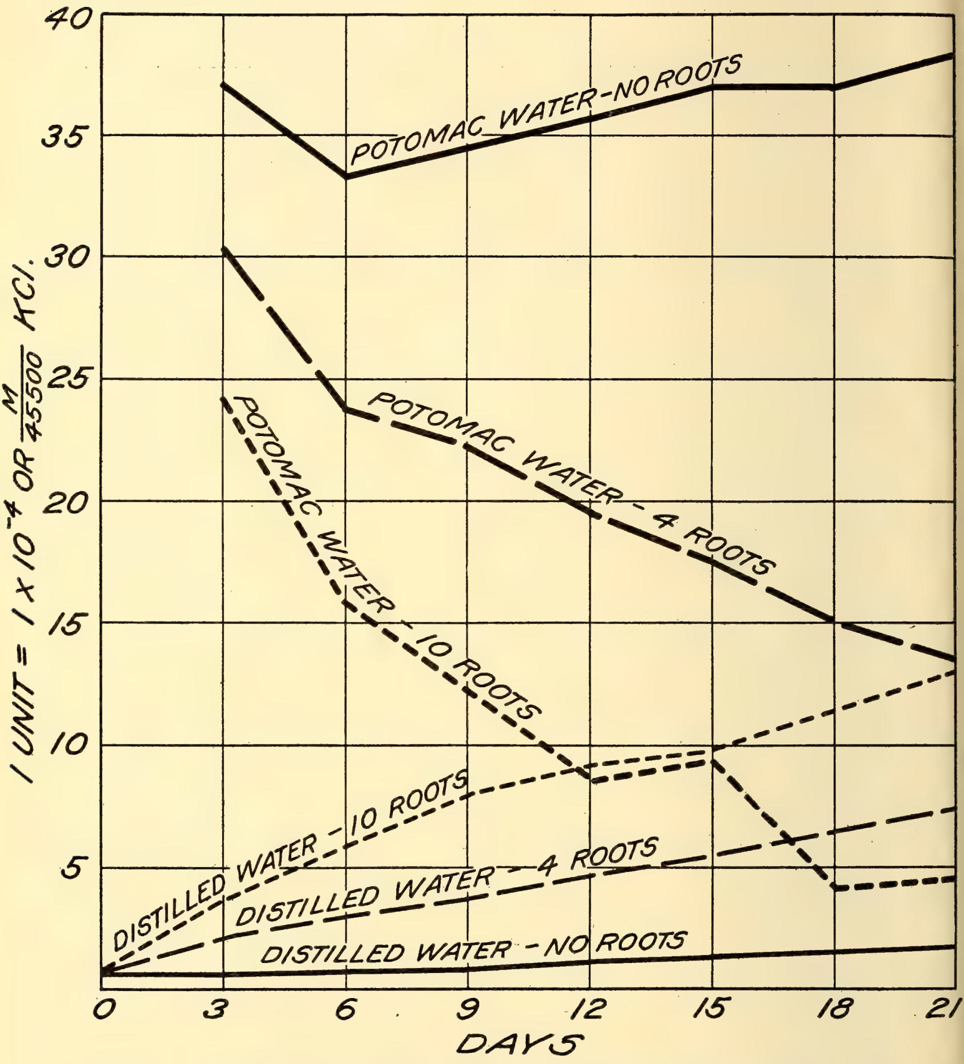

Fig. 1. Changes in the conductivity of distilled water and of Potomac water due to their action on lupine roots.

lytes as the distilled water culture containing 10 roots, and only about twice the quantity found in the distilled water containing 4 roots.

The distilled water check containing no roots showed a slight though steady rise, due largely perhaps to the gradual solution of the glass container. The Potomac water check showed more irregularity in its behavior due to causes not determined, the result being a gradual rise in conductivity following a rather abrupt fall early in the experiment. This first abrupt fall is possibly due to the breaking down of $CaN_2(CO_3)_2$ with the formation of insoluble $CaCO_3$.

In order to make the general features of the experiment clearer, the accompanying curves have been platted. On the axis of ordinates one unit equals a conductivity of 1×10^{-4} corresponding to a solution containing $M/45,500$ KCl. On the axis of abscissas five space units equal an interval of three days. (Fig. 1.)

It has now been shown that water of a rather high degree of purity is harmful to the roots of lupine seedlings suspended in it; that the growth rate of these roots rapidly falls off and at the end of a period of three days is almost zero; that at the same time the roots continually give up electrolytes to the water, and the causal connection between the loss of electrolytes by the roots and their falling growth rate is regarded as almost certain. The check cultures carried on in Potomac water containing small amounts of the common salts present in the soil show a probably normal growth rate accompanied by an absorption of electrolytes from the river water.

The question was next raised whether with the addition of soluble substances in quantities sufficient to make the water cultures osmotically equivalent to Potomac water, the growth rate of the plants would be favorably affected. Accordingly, cultures of cane sugar, calcium chloride and sodium chloride were set up having an osmotic value equal to that of Potomac water.

TABLE IX

ACTION OF ISOTONIC SIMPLE SOLUTIONS

Description of medium	Growth rate		Electrical conductivity at end of 48 hrs.
	First 24 hrs., mm.	Second 24 hrs., mm.	
Distilled water + cane sugar	13.0	4.5	1.39×10^{-4}
Distilled water + sodium chloride	19.0	5.5	20.8×10^{-4}
Distilled water + calcium chloride	27.0	22.0	21.7×10^{-4}
Distilled water	12.5	5.0	1.22×10^{-4}

This experiment seems to show that the harmful action of distilled water as reflected in the growth rate is not due to the aggregate difference in osmotic pressure between the cells of the roots and the external medium, since the sugar introduced equals the osmotic aggregate of the river water. This substitution seems to leave the water with harmful properties undiminished, although sugar in itself at this concentration can hardly be called distinctly harmful. As was observed by Loeb (30) in his work on *Gammarus*, one finds here no marked improvement following the addition of the sugar. Indeed, the main features of the experiment strikingly resemble those of the check culture in which distilled water alone was used. Leaching was not hindered by the presence of the sugar but was perhaps somewhat accelerated.

The addition of sodium chloride in quantity osmotically equal to the dissolved material in Potomac water exerts a marked beneficial action in the first 24 hours, but this seems to be lost during the second day. It is obvious that although the presence of this salt protects the roots to a certain degree, the protection is of a decidedly temporary nature. It also confirms the conclusion based on the action of the sugar solution that simple aggregate osmotic pressure is not the most important feature in the case.

The addition of calcium chloride in osmotically equal quantity produces a strikingly different result. The growth rate assumes an apparently normal character throughout the course of the experiment, the peculiar "distilled water" action being absent. Since the Potomac water approaches in conductivity to a $M/1{,}000$ KCl solution, giving a conductivity under the conditions of these experiments of about 37×10^{-4} reciprocal ohms as against 44.5×10^{-4} like units for $M/1{,}000$ KCl, it appears that the sugar solution behaves essentially like distilled water in showing an increasing conductivity on account of the extraction of electrolytes from roots. This assumes that the sugar is not significantly conductive under these conditions. It is also clear from the higher resistances that roots absorb electrolytes from the isotonic solutions of NaCl and $CaCl_2$ in a way comparable with the absorption from Potomac water in the foregoing experiments (p. 266). In the case of these isotonic salt solutions, it is probable in the absence of considerable quantities of other electrolytes in the distilled water, that the absorption of NaCl and $CaCl_2$ takes place. If such is the fact, the growth rate during the second day should reflect to a certain degree the effect of such absorption. If this assumption is justified, the absorption of NaCl is less favorable than the absorption of $CaCl_2$. On the other hand this difference in effect may be due to the different effects these salts have on the leaching of the cells. There seems to be good reason for thinking that abundant leaching of certain classes of compounds present in the cell would take place in the presence of but a single salt in the exterior medium.

The experiment above described was repeated with a number of variations.

Table X

Action of Isotonic Simple Solutions

Description of medium	Growth rate		Electrical conductivity at the end of 48 hrs.
	1st 24 hrs., mm.	2d 24 hrs., mm.	
Distilled water + cane sugar	13.0	5.0	1.0×10^{-4}
Distilled water + NaCl	17.0	9.0	18.9×10^{-4}
Distilled water + $CaCl_2$	24.0	12.0	18.9×10^{-4}
Distilled water + $Ca(NO_3)_2$	22.0	15.0	18.9×10^{-4}
Distilled water + ½ equiv. NaCl ½ equiv. $Ca(NO_3)_2$	23.0	16.0	18.9×10^{-4}
Distilled water	15.0	8.0	1.25×10^{-4}
Potomac water	24.0	22.5	15.4×10^{-4}

Again the inefficiency of cane sugar as a means of improving physiological conditions is marked. The half-way benefit of NaCl again appears and the great help due to the presence of Ca salts is apparent. That it is due to the action of the Ca ion is clear from the fact that the benefit is derived from both Ca salts in spite of the fact that different anions accompany Ca. The falling off of the growth rate in the Ca-containing solutions during the second day may indicate among other possibilities that the Ca solutions fail to supply necessary needs for more than a short time or that they may fail to check the undetected leaching of small quantities of necessary substances. When one half of the osmotic equivalent of Potomac water is supplied by NaCl and one half by $Ca(NO_3)_2$, the record made by the roots is essentially like that produced by the Ca salt alone in full equivalent. This may mean that the Ca action is so marked even at this very considerable dilution (nearly equivalent electrolytically to $M/2,000$ KCl solution) as to cover up the less favorable action of the NaCl present and give it in effect a Ca effect.

The check cultures in distilled water and in Potomac water serve to illustrate the great difference for *Lupinus albus* between the distilled water and Potomac water as culture media. They show clearly that distilled water should be used with caution in laboratory work as a check medium intended to present a norm of plant action. Potomac water certainly furnished more favorable and natural conditions for root growth in the plant here concerned than distilled water. It should be noted, however, in this connection that all plants are not equally sensitive to injury from this source, it having been shown by True and Bartlett (39) that Canada field peas make a fairly healthy growth in distilled water in spite of the fact that they lose a considerable quantity of electrolytes to the outer medium.

Summary of Results

It appears probable from the results given in the course of this paper that the problem of injury by distilled water is not a simple one capable in all cases of a like explanation. In some cases, distilled water obtained from apparatus having copper surfaces exposed to contact with the water undoubtedly derives certain toxic properties from minute traces of copper. In other cases doubtless it is possible for other harmful impurities to find their way into the product, but after the action of all the impurities has been accounted for there still remains a residuum of harmful action due to no known type of impurity. This mode of harmful action seems to be most marked in water which shows the highest resistance to the passage of the electric current.

It is shown here that those samples of distilled water which show the highest resistance are in general more harmful to lupine roots than waters containing a large quantity of electrolytes. It is likewise shown that these same samples of water withdraw electrolytes from the tissues of the roots when they remain in the water. This leaching of electrolytes is shown to be the probable mechanism by means of which purer samples of distilled water exert their harmful action on the roots. This extraction by distilled water is regarded as but a special case of the general type of injury wrought on cells by unbalanced solutions whereby certain necessary constituents, undoubtedly in part inorganic, are dissociated from their proper attachments in the complicated chemical and physical mechanism of the living cell. The distilled water seems to withdraw material required for the maintenance of the efficient action of the protoplasmic limiting membranes with the result that the permeability of the cells is increased, and a further dissociation of electrolytes from their points of combination in the proteids, and other chemical structures of the cell ensues. These dissociated electrolytes escape from the cell and increase the conductivity of the distilled water. When a calcium salt is added to the distilled water sufficient to make it osmotically equal to tap water, this dissociating power of the distilled water over the proteids and other chemical mechanisms of the cells is largely undeveloped, and the chemical integrity of the cells is protected in some way not known.

This report is preliminary in its nature and is to be followed at a future date by a further contribution reporting the results of work now under way.

Bureau of Plant Industry,
U. S. Department of Agriculture.

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SOME OBSERVATIONS ON THE FORMATION OF THE CAPILLITIUM AND THE DEVELOPMENT OF PHYSARELLA MIRABILIS PECK AND STEMONITIS FUSCA ROTH[1]

G. R. Bisby

WITH PLATE XXIV

While examining preparations of *Physarella mirabilis* with a view to a study of nuclear phenomena, certain observations were made on the development of the capillitial threads so numerous in this species. In view of the fact that Physarella has not heretofore been studied cytologically; and, further, since the formation of the so-called solid capillitium is still a matter of discussion, it seems worth while to publish at this time the results so far obtained. While the study was in progress, some fruiting specimens of *Stemonitis fusca* were secured, from which supplementary data were obtained.

The literature pertaining to the capillitia of Myxomycetes has been carefully summed up by Harper ('00, '14*b*) and by Harper and Dodge ('14*a*), so that it is unnecessary to give here more than a brief glance at earlier investigations. Harper, in his papers on the subject, has described thoroughly the formation of capillitium in certain species in which it is distinctly hollow, and was able to corroborate and add much to Strasburger's ('84) earlier account of the process of formation. In his paper on cleavage in a recent issue of this Journal, Harper gives incidentally some data regarding the capillitium of *Didymium melanospermum*, but does not dwell upon the methods of its formation.

Materials and Methods

Pieces of well rotted wood were collected in Prospect Park, Brooklyn, in November, 1913, and placed in covered battery jars. Upon this wood developed several species of Myxomycetes, the plasmodia of Physarella being especially abundant. Sporangia in large numbers were formed repeatedly, yet sufficient vegetative plasmodia usually remained to continue the culture. These developing sporangia were fixed in various stages of development in the Flemming-Strasburger medium solution, and stained principally with Flemming's triple stain.

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[1] Brooklyn Botanic Garden Contributions, No. 8.

The development of sporangia in cultures usually began during the afternoon and was completed by the following morning. Some little variation was observed as to the hour of maturity of certain fruiting groups, due possibly to somewhat unusual conditions of light, heat, or moisture. It was curious, however, to note that the various forming sporangia of a group developed almost simultaneously, in spite of the fact that all connection between them was early lost, and that some were at the edge of the water in the bottom of the jar, while others were formed on higher and apparently much drier portions of the wood. Mature sporangia were, however, perfectly normal in any case.

Capillitium Formation in Physarella mirabilis

A mature sporangium (see figure 15) in this species contains a large number of more or less branched capillitial threads radially arranged. These threads are smooth, except for an occasional small spinous projection, and at first glance appear solid, except where they broaden out to constitute a lime knot. These knots are numerous, and vary in size from that of a barely perceptible swelling in a capillitial thread, to large spicules extending from side to side of the sporangium.

When the plasmodium first begins to form lumps where sporangia are to occur, the protoplasm presents no essentially different appearance from that of the vegetative stage, save that much of the extraneous substances has been extruded and left along the somewhat slimy trail; the same bubbly appearance is presented. Before long, however, spaces appear in the protoplasm, into which waste substances, largely lime granules, are excreted. In sections of the protoplasm as it becomes more finely granular, fine tubes can be detected, sometimes connecting the knot spaces with the exterior, and sometimes without any perceptible attachment to a developing lime knot. The membrane surrounding the tubular opening is continuous with the membrane surrounding a knot space, if any of the latter be in connection with this opening.

Such a condition is shown in figure 1; the lime knot and capillary tubules connected with it are bounded only by a plasma membrane continuous with the membrane at the external surface of the protoplasm. In this figure the protoplasm has shrunken slightly away from the sporangium wall. This shrinkage is undoubtedly artificial. The lime knot is nearly filled with cystolithic granules of calcium carbonate; in this instance, the acids of the fixative apparently did not penetrate sufficiently to dissolve out the lime. In the figures following (figures 2–4, 11) representing succeeding stages, the calcium carbonate appears to have been largely removed and only a small amount of substance remains in the lime knots. In young stages, the tubular spaces which extend through the protoplasm and which define the location of future capillitial threads, usually appear empty and are rendered visible only by very careful focusing. They are usually of a very narrow diameter; most of the mature threads, for that matter, are also much attenuated. The capillitial space in the center of figure 1 is, however, slightly wider than usual and contains some stainable substance, possibly lime. The sporangial wall in this and certain other figures presents an altered appearance due to the removal of the lime naturally present. Two aggregations are shown, however, in the wall of figure 1 which are interpreted as areas in which lime has been present.

Figure 2 represents a somewhat later stage of capillitium formation. The protoplasm is seen to be in contact with the forming thread, a plasma membrane being evident only where the lime knot, from which the contents have been largely dissolved, has shrunken away. The sporangial wall in this figure is rather thick, and its inner surface is in direct continuity with the walls of the thread, while the thread itself is in turn continuous with the walls of the lime knot. This capillitial thread in figure 2 is clearly hollow save for an attenuated portion near the periphery of the sporangium, where no lumen is evident.

The threads are by no means all formed simultaneously. Spaces without deposition may be found in sections showing capillitial threads which are nearly mature.

Figure 3 shows similarly the continuity of the thickened walls of the lime knot and those of the threads. In this case a more advanced stage is figured, for the bounding walls are much thicker than in the previous figure. Shrinkage of the lime knot has not occurred, although its contents have been largely removed.

In figure 4 the hollowness of the narrow thread is not apparent. Here is shown further the connection of the capillitial threads and the lime knots, as well as a branching thread. Figure 5, from an older sporangium, shows again the connection of thread and exterior. The hollow nature of the thread is here again plainly apparent where it widens out slightly. In this figure the protoplasm appears not at all shrunken, being in intimate contact with both capillitial thread and sporangial wall.

Figure 6 represents a small section of material collected in the morning shortly before the time of complete maturity of the sporangia. In some sporangia of the collection at this hour, cleavage had progressed to a considerable extent; while in the majority of cases cleavage is not so evident, the protoplasm being still in close contact with the thread, in the manner shown in the figure. Figure 7 does indeed indicate a case at this stage in which there is a slight space between the thread and the plasma membrane, and similar cases may in fact be found at other stages. I have interpreted such cases, when occurring at some time prior to cleavage, as arising from a slight shrinkage of the protoplasm away from the threads, due probably to fixation. At any rate, this earlier shrinkage should be clearly distinguished from the normal cleavage furrows which later follow the capillitial threads and which have been interpreted below as normal occurrences. The nuclei present an interesting appearance at this stage for, as is shown by one in figure 6, many are surrounded by a vacuole-like space. Harper points out ('14*b*, p. 133) this same phenomenon in Didymium; and interprets it as "hardly due to shrinkage in fixation, since they are scattered among other nuclei which show no such peculiarity."

CAPILLITIUM IN RELATION TO CLEAVAGE IN PHYSARELLA

Although the threads may be found in contact with the cytoplasm until a short time prior to cleavage, at the first suggestion of that phenomenon there is no doubt but that this contact ceases to exist. Harper has elaborated this point in his Didymium paper. Figure 8 shows an early stage of cleavage, in which every forming furrow is plainly associated with one or more capillitial threads, which appear in cross or oblique section in the preparation. The threads all appear to be hollow; that this appearance is not an artifact is attested by numerous observations on other material. At the upper right in figure 8 is a thread which has not yet been surrounded by a cleavage furrow. However, a slight space, as is commonly found at this stage, surrounds the thread. Conditions in the left part of figure 8 portray a slightly more advanced state in that the cleavage furrows are of a greater diameter. Near the center of this figure cleavage has not actually been instituted, but the cytoplasm presents a more hyaline appearance, suggesting very clearly that cleavage in this place is about to result.

In figure 9 is represented a stage of cleavage slightly more advanced, in which the nuclei are in the equatorial plate stage of division, and each is surrounded by a hyaline space. The longitudinal view of the thread in this figure shows plainly its hollow nature. The manner in which cleavage furrows follow the threads is suggested by this figure, although it is difficult to picture the irregular fissures passing as they do at right angles to the axes of the capillitial threads, as well as up and down them.

Figure 10 shows a still later stage in cleavage. The large piece of spore-plasm shown in the figure is bounded here and there by capillitial threads in close proximity. Many of the threads lie, in fact, in bays or deep indentations of the protoplasmic mass, suggesting their intimate association with the inauguration of the cleavage furrows. A shrunken lime knot is represented in cross-section at the upper left part of the figure.

Figure 11, from a nearly mature sporangium, shows again the continuity of the inner portion of the sporangial wall and the exterior boundary of a larger lime knot, cut somewhat diagonally. The lime contents of both knot and wall have been largely removed by the action of the fixative.

Figure 12 was drawn from a mature sporangium crushed in a drop of water on the slide. It shows a mature thread, plainly hollow and not solid, as might be supposed from a hasty examination, of somewhat varying diameter, and two lime knots, the larger of which is small compared with the size of an average knot. A spore is shown adjacent for comparison of size. Within the knot, as is in fact always the case in Physarella, are many granules of calcium carbonate, rather uniform in size and shape. When these are dissolved in acetic acid, the knot presents the same essential appearance as it does in sections of fixed material.

Figure 13 represents a mature capillitial thread. As is shown, branching occurs; while the surface is smooth save for the "spines" occasionally present. This thread is hollow, as is represented in the figure, but this condition is by no means clearly apparent in every mature capillitial thread. Chloroiodide of zinc solution, with which they stain yellow, often serves to bring out more clearly the hollowness of these threads.

Figure 14 is another preparation from a mature sporangium, the figure being a surface view of a portion of the sporangial wall. A small but definite area is shown densely charged with lime granules, while in the surrounding wall are embedded more or less isolated lime granules. The larger lime knot undoubtedly corresponds to the knots shown in section in figures 1 and 11. The darker spots in figure 14 represent aggregations of yellow coloring matter. It may be noted here that the plasmodia of *Physarella mirabilis* are bright yellow; the hollow stalk of the mature sporangium is reddish; while the spore bearing portions are, to the naked eye, grayish-yellow. Each lime granule in a knot or in the sporangial wall has also a yellowish tinge, and this color seems to dissolve out in water, leaving the granules nearly colorless after a time. The capillitial threads also have a yellowish cast when viewed under the microscope.

Figure 15 is a partially diagrammatic drawing of three-fourths of one of the mature, somewhat funnel-formed sporangia, on its hollow, cylindrical stalk, showing the relation of the various parts.

The Capillitium of Stemonitis fusca

While the work on Physarella was in progress, fruiting material of *Stemonitis fusca* was collected from one of the cultures. It was not long after the emergence of the white plasmodium from the interior of the wood, that a heaping up of the protoplasm occurred, defining the sporangial groups. Complete development from the first appearance until maturity occupied only the twelve hours from noon to midnight. About two hours after the sporangia in the fruiting groups had attained their typical size and shape, the brown tint of maturity was apparent.

The classical example of the so-called solid capillitium is that of Stemonitis. The arrangement of the intricate system of capillitial threads in this species is a familiar picture. The hollow stalk continues as a central columella to the very summit of the sporangium. From this columella branches off at right angles a profuse system of rather coarse, branching threads, which terminate sub-peripherally just within the fragile, often evanescent sporangial wall in a delicate, anastomosing, capillitial sac.

In longitudinal sections of very young stages, the hollow stalk is seen extending as a comparatively large, cylindrical cavity from the very base of the fruit body up to near its summit. The plasma membrane surrounding this central cavity secretes the hollow stalk up which the protoplasm climbs to form finally the sporangium along its upper portion. Figure 16 shows in median section the upper portion of such a central axis, cut somewhat obliquely. At its very apex, the irregular and rather tubular space in which the columella is formed appears broader than it is below, where deposition of wall substance has begun. In the lower part of figure 16 the somewhat shrunken and diagonally cut columella has attained some degree of thickness from deposition of wall substance from the plasma membrane. Into the upper portion of this space may be noted the intrusion of finger-like processes of protoplasm, while some irregular masses of protoplasm lie detached within the cavity itself. Indeed, one quite commonly notes in sections the presence of protoplasmic masses inside the columellar cavity, even in much older stages. The figure illustrates quite clearly just how their presence in such situations is to be explained, arising as they do from fragments detached from above by the ever advancing columellar apex. This point will be considered in some detail in the general discussion which follows.

Just above the apex of the cavity in which the stalk is being formed is seen the much vacuolated protoplasm which marks the region into which the columella is soon to advance. Denser areas lie still further beyond; while still other dense areas are shown at the sides of the upper portion of the space. Extending upward at a slight angle from the rising columellar cavity may be noted a tubular space. Careful focusing at this part of the preparation reveals in fact a profusion of such narrow spaces, radiating in an upward direction from the apex of the large cavity and extending in some cases even to the periphery of the mass of protoplasm. Occasionally it can be seen that such tubular spaces are directly connected with the main large columellar cavity, the plasma membrane of the one being continuous with that of the other.

Forming capillitial threads may be noted in quite an early stage of the sporangium. Not, however, until the structure had assumed its final form, in the fruiting material studied, were the capillitial threads observed in any abundance. The method of deposit of capillitium in *Stemonitis fusca* appears to be in entire agreement with the process as found in Physarella. Figure 17 shows an early stage in which the tubular space in the still foamy protoplasm is bounded only by a thin membrane, no wall deposit being as yet apparent. I have not attempted to show in great detail the various degrees of thickening of these developing threads, instances of which are rather common in the preparations. Figures 18, 19 and 20 show this phenomenon incidentally, and perhaps as convincingly as a more detailed series of drawings would.

Figure 18 represents the attachment of a capillitial thread to the columella. The thread has here attained some thickness, as may be observed further away from the central columella where the thread widens somewhat. At this wider portion the wall appears somewhat wrinkled or irregular. Near the columella the thread is of sufficient degree of attenuation to obscure the lumen. The protoplasm in this instance is in close contact with the thread. The expanded attachment of the capillitial thread to the columella is of some interest, since it is evident that the broader attachment is a continuation of the outer portion of the thickened central columella. So far as my own observations have gone, I have not found the lumen of the columella to be continuous with the lumen of the capillitial thread, which point is in agreement with De Bary's findings.

Figure 19 shows a cross section of material in a similar condition; here, however, the protoplasm has been shrunken away from the thread and half the columella, possibly by the initiation of cleavage. Within the columella may be noted the presence of some stainable substance, probably protoplasm.

Figure 20 represents a case in which cleavage is just beginning from the surface of the sporangium. The nuclei are in the equatorial plate stage, and the protoplasm is distinctly more finely granular and richly stainable at the periphery, perhaps due, as Harper suggests, to contraction of sporeplasm and extrusion of water. As is shown in the figure, the reticulations that form the net-like capillitial sac of the mature sporangium have been already formed just within the outermost portions of the protoplasm. Deeper down within the more foamy protoplasm the threads are still, in some cases, clearly in contact with the cytoplasm. In most cases in this preparation it was clearly evident that the threads are of a hollow nature. Although any relation to cleavage is not definitely shown in this figure, it is apparent from a study of the sections that here also the cleavage furrows are influenced and defined to a considerable degree by the capillitial threads, just as in Physarella.

As is well known, the sporangial wall in Stemonitis is very fragile and eventually disappears. In younger stages it may be noted as a thin bounding membrane; while from older or mature sporangia it is usually missing. In figure 21, from a preparation similar to the one from which figure 20 was drawn, this outer wall is shown, broken and removed from contact with the protoplasm. A very delicate, connecting capillitial thread is to be seen in figure 21, its attachment to the sporangial wall quite comparable to that already examined in Physarella.

Figure 22 is a high power drawing of a mesh of the inner portion of the mature dried capillitium. As is indicated by the dotted line, a portion is clearly hollow; while other parts, due to their dark color and narrow diameter, did not present an appearance that could render possible a statement that they were hollow. Of course an appearance simulating hollowness can easily be obtained if a thread is slightly out of focus. There is, however, no doubt but that, though the lumen may be at times extremely capillary, the most of these threads are really hollow; a focus on a bent "knee" of an anastomosing thread, for example, often clearly demonstrates this point.

General Discussion

The term "vacuoles," as applying to the openings in which the capillitial threads are to develop, would seem to be misleading in the case of the two species under discussion, since these openings in question appear to be quite commonly, if not indeed invariably, invaginations from the sporangial surface, or from the plasma membrane bounding the columella. It is of course quite likely that these more or less tubular openings in the protoplasm are due to some tensions arising in the viscid mass; the fundamental causes resulting in this and related phenomena are, however, obscure. Much easier to understand, it seems to the writer, is the process of deposition of the fine, smooth capillitial threads.

Taking first the case of *Physarella mirabilis*, tubular capillary spaces appear in early stages of sporangial development. These spaces are no doubt filled at their beginning with watery sap and aqueous waste, connected as they are with lime-knot spaces and opening upon the developing outer membrane. From the plasma membrane surrounding these capillitial primordia, wall substances are next secreted. I am as yet unable to state positively whether the thin wall deposited early in capillitium formation is folded or actually pushed inward by subsequent external deposits from the surrounding plasma membrane; or whether the tubular cavity gradually increases in size, deposition thus resulting in a constantly increased total diameter of the capillitial threads. I am inclined to think that the former process obtains to a certain extent and that actual diminution in size of the lumen of the capillary tubules sometimes consequently results. The substance secreted to form the walls of these hollow threads is presumably plastic for some little time after formation. Furthermore, there may undoubtedly occur a wrinkling or folding of the thread during the process of drying, particularly in regions where it is comparatively broad. This latter appearance is more frequent in *Stemonitis fusca*. However, in any case, as has been previously stated, there can very frequently be observed within the thread a lumen of greater or lesser diameter. I know of no good reason why practical solidity should not occur in certain cases; such, for example, as would result from a deposit in a tubular space of extremely capillary proportions.

It is further shown beyond doubt in the case of Physarella the same method of deposit obtains in the formation of walls about both lime knots and capillitial threads, as that seen in the formation of the inner part at least of the sporangial wall. The first few figures show clearly the continuity of the boundary of those parts. Although the external surface of the outer peridium appears thicker, presumably from hardening through exposure to the air, I have no doubt but that the whole sporangial wall is of similar character and deposited by protoplasmic secretion.

The large amount of lime in the mature sporangium of Physarella suggests an approach to the condition obtaining in *Fuligo varians* in regard to the amount of lime present. The old analysis of Reinke and Rodewald ('81) credits that species of Fuligo with 27.7 per cent of calcium carbonate, and 5.33 per cent of calcium in combination with higher fatty acids. The absorption, deposition, etc., of this calcium carbonate in these Myxomycetes furnishes an interesting problem, since, as is well known, $CaCO_3$ is soluble only in 100,000 times its own weight of pure water. It is further curious that some species should provide themselves with such large quantities of calcium carbonate, while others apparently lack it entirely.

The formation of the columella in *Stemonitis fusca* takes place by means of a progressive deposition of wall material along a cylindrical space that reaches from the base of the young fructification upward through the rising mass of protoplasm. The irregular radiations and extensions of the apical part of this space resembles somewhat the condition as figured by De Bary ('87) for *Stemonitis ferruginea* (p. 432, figure 186, a) in which the upper portion of the columella is pictured as frayed out in a brush-like fashion. The exceedingly vacuolar appearance of the protoplasm above the columellar space (see figure 16) suggests both the rapid pushing up of slender, tubular invaginations from the apex of the space itself, as well as the final incorporation into the columellar space of the vacuoles that lie in its upward path.

It has already been pointed out that some protoplasm is regularly left within the columellar space, cut off from the mass above the columella by the upward pushing, slender invaginations and vacuolar cavities which are characteristic of this area apically from the tube. In younger stages of columella formation, the protoplasm included in its hollow interior shows some evidences of continued activity, although often broken up into very small, rounded fragments. In older stages, however, its appearance suggests a much less active condition; and it probably ultimately dies.

In Stemonitis the same essential phenomena in regard to capillium formation undoubtedly obtain as those described above for Physarella. Views of early stages of the spaces in which are to be secreted the capillitial threads show an appearance of considerable irregularity and anastomosis, and they are with difficulty traced through the foamy protoplasm (figure 17). Later stages show the threads with more or less thickening deposited adjacent to the plasma membrane. Ordinarily, careful focusing on thin sections reveals clearly the tubular character of these threads. The attachment of capillitial threads to the central columella displays a condition somewhat comparable to the attachment to the sporangium wall in the case of Physarella. It is noteworthy in Stemonitis that the threads are evidently in connection with the exterior deposition upon the columella, pointing to the fact that the threads are formed by a deposition no different from that which forms the columella; nor indeed from the wall deposit itself, as figure 21 evidences. The further fact that in the cases figured (figures 18 and 19), the lumen of the thread is not continuous with the lumen of the hollow columella shows that in these instances at least, the capillitial cavities were started to be formed some time after the columella wall deposit had attained some degree of thickness. If the capillitial tubules should begin to form as early as columella deposit, the lumen of the two should undoubtedly be continuous.

The formation of the reticulated, capillitial sac just within the exterior protoplasm of Stemonitis is at first glance a puzzling phenomenon. This would obviously result in a layer of spores being formed outside the main capillitial network; in fact, a careful examination of a fresh, mature sporangium shows that such is indeed the case. It is evident that this rich development of a layer of capillitial threads just within the peripheral protoplasm must result from a very rapid secretion of wall substances from the rapidly maturing external protoplasm. Only comparatively few of the very delicate threads which connect this anastomosing network with the external wall apparently have an opportunity to form, so quick are these changes in the peripheral portion of the sporangium.

Contrasting the sporangial walls of the two species under discussion, it seems clear that the thickness of wall deposit must depend to some extent upon the length of time the peripheral protoplasm remains in a relatively quiescent state. In Physarella, external secretion apparently continues during a considerable portion of the time of development of the sporangia. In Stemonitis, on the other hand, cleavage begins at the exterior very shortly after the heaping protoplasm attains its final shape, and in consequence the sporangial wall reaches only a meager development.

Some mature capillitial threads in *Stemonitis fusca* are somewhat flattened; some are somewhat irregular in shape, especially at the meshes; some present a wrinkled or crumpled appearance, as before mentioned. In some cases the occurrence of solidity is hard to disprove; but studies of many sections and of mature capillitia indicate without any doubt that hollowness of the threads is in reality very common.

The smoothness of the exterior of the capillitial threads in both species studied is in striking contrast to the spirally thickened and otherwise marked, distinctly hollow threads of certain other Myxomycetes, such, for example, as Trichia. But in the essential features of the process, the phenomena connected with the formation of the two types of threads are regarded as entirely similar, consisting in both cases in the deposition of hollow threads by plasma membranes lining tubular capillary spaces. In Trichia, however, the capillitial cavities are of relatively large diameter; while in the two species described in this paper, they are of very narrow diameter. The fact that in Trichia and similar cases, the capillitium starts in vacuoles in the interior of the protoplasm, while in Stemonitis and Physarella these spaces apparently originate as invaginations of the external plasma membrane or that lining the capillitial or columellar cavities, is undoubtedly of fundamental importance.

Cleavage in these two forms under consideration is decidedly progressive. This shows particularly strikingly in cross sections of young sporangia of Stemonitis, in which cleavage will often be seen to involve at one time only a small sector of the peripheral protoplasm. I am unable, however, to add anything new to the valuable observations made along these lines by Professor Harper ('00, '14*b*).

I am indebted to Dr. Olive, under whose supervision this work was carried on, for suggestions and criticisms freely given during the course of these investigations.

Summary

1. The capillitium of *Physarella mirabilis* and of *Stemonitis fusca* is formed within tubular capillary spaces. In both cases these spaces are formed as invaginations into the protoplasm, and are not considered to be vacuoles. From the plasma membrane lining the tubular spaces occurs a progressive deposition of substance to form the walls of the capillitial threads. This deposit is continuous with that forming the sporangial wall; and, in Stemonitis, as well with that which forms the columella wall, to which the radiating, tubular threads are attached. In Physarella, a continuous wall also exists, bounding capillitial threads and lime knots, when they are in connection with each other.

2. The protoplasm remains in contact with the capillitial threads until a short time prior to cleavage. Cleavage furrows then appear, which follow the threads and which are thus determined to some extent by the capillitium.

3. The mature capillitial threads of these two forms are smooth (in Physarella an occasional spinous process occurs), and a careful examination shows that a majority of the threads are hollow and not solid as is usually stated. Where seeming solidity does occur, this is interpreted as due either to an actual collapse of the tube when in a plastic condition, or else in reality to the obscurity of the lumen owing to the fineness or opacity of the threads.

4. The columellar cavity in *Stemonitis fusca* pushes up from the base of the young fructification through the heaping protoplasm as a more or less cylindrical space. Progressive deposition of wall substance from the plasma membrane surrounding this space forms the resulting thick-walled, tubular columella.

5. The delicately anastomosing reticulations of the mature capillitial sac in *Stemonitis fusca* are formed subperipherally, just within a layer of peripheral protoplasm. In earlier stages, these reticulations are attached by a few fine capillitial threads to the fragile and evanescent sporangial wall.

Brooklyn Botanic Garden, Brooklyn, N. Y.

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EXPLANATION OF PLATE XXIV

The drawings, except figure 15, were made with the aid of the camera lucida, and drawn at the level of the stage. Zeiss compensating oculars 2, 6, 8 and 12 were used, together with Zeiss 2 mm. apochromatic objectives NA 1.30 and 1.40.

Figures 1–15, *Physarella mirabilis.*

Fig. 1. The early appearance of capillitial spaces, and a young lime knot. The sporangial wall, from which the protoplasm has somewhat shrunken, is already rather thick. × 750.

Fig. 2. The capillitial thread, sporangial wall, and lime knot all have a common continuous boundary, which has in this instance attained an appreciable thickness. Shrinkage has occurred from the wall bounding the lime knot. × 1,000.

Fig. 3. A somewhat later stage, with still thicker wall about capillitium and lime knot. × 1,500.

Fig. 4. A preparation similar to figures 2 and 3, but showing shrunken lime knot and branching capillitial thread. × 1,000.

Fig. 5. A capillitial thread and sporangial wall again showing continuity of bounding membranes. × 1,500.

FIG. 6. Almost mature capillitial threads in which the lumen is not evident. × 1,000.

FIG. 7. A stage approaching maturity, in which a space is shown surrounding the capillitial thread. × 1,000.

FIG. 8. The cleavage furrows are limited by the capillitial threads, which appear in cross-section in the preparation, or else are cut somewhat obliquely. × 1,000.

FIG. 9. A preparation taken from a similar stage, showing a cleavage furrow following in part the hollow capillitial thread. × 1,000.

FIG. 10. Another similar, but somewhat older, stage of cleavage. × 1,000.

FIG. 11. Showing the attachment of the sporangial wall and a lime knot. × 750.

FIG. 12. A mature capillitial thread, containing two small lime knots; and a spore shown for comparison. From a mature, crushed sporangium. × 750.

FIG. 13. Another capillitial thread, showing branching and the occasional spine-like projections. × 1,000.

FIG. 14. Surface view of a portion of the wall of the sporangium, showing a large lime knot, small lime granules, and the yellow (shown as darker) pigment granules. × 1,000.

FIG. 15. Diagrammatic drawing of a mature sporangium, showing expanded base, hollow stalk, funnel-shaped sporangium, sporangial wall, and the radially arranged capillitium and lime knots. × about 20.

FIGURES 16–21, *Stemonitis fusca.*

FIG. 16. Upper portion of the space in which the columella is to be formed; showing thin columellar wall, protoplasmic inclusions, and the vacuolated area in the path of the rising axial cavity. × 250.

FIG. 17. Young stage in capillitium formation; showing a space in which no perceptible deposit of wall substance has as yet been made. × 1,000.

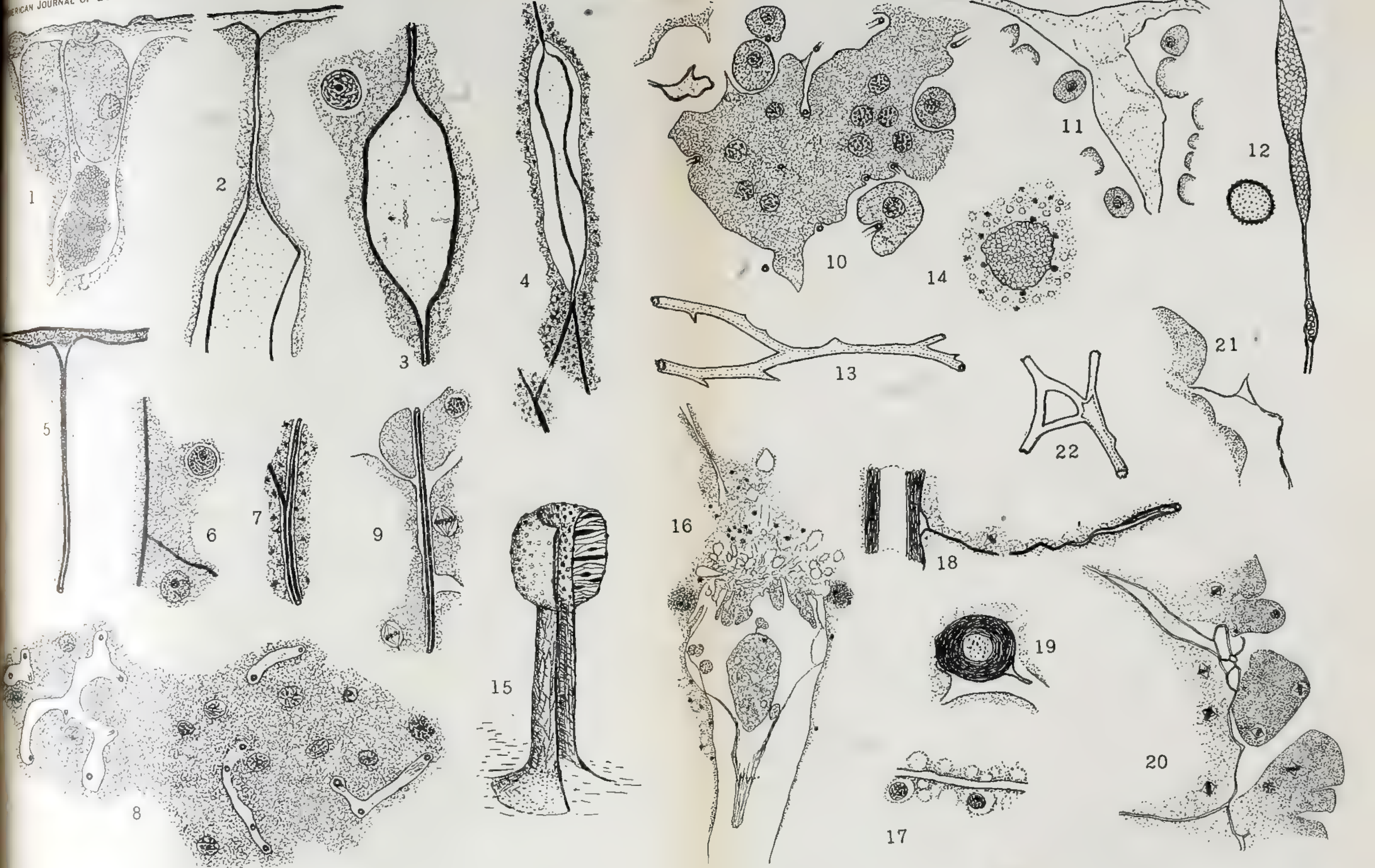
FIG. 18. Late stage in the development of the capillitium, showing attachment to the columella. × 750.

FIG. 19. Similar condition to figure 18, but showing columella in cross-section. × 750.

FIG. 20. A preparation from the peripheral portion of a sporangium, showing cleavage begun; together with a small fragment of the delicate, subperipheral, anastomosing, capillitial sac. × 750.

FIG. 21. A portion of the fragile, evanescent sporangial wall, showing a small capillitial thread attached. × 1,500.

FIG. 22. Small portion of capillitium, from a mature sporangium, showing anastomosis, and the hollow character of one thread. × 1,500.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BISBY: PHYSARELLA MIRABILIS AND STEMONITIS FUSCA.

NEW SPECIES OF GREEN ALGAE

EDGAR NELSON TRANSEAU

In the course of my study of the periodicity of occurrence and reproduction of the algae of eastern Illinois during the past several years, a number of undescribed forms have appeared in the collections, of which the following are now sufficiently well known to be described. In accordance with the rules of the Vienna Congress a Latin as well as an English diagnosis is given in each case. Camera drawings from the type material are added together with notes on the relationship and occurrence of each of the new forms. A part of these species have been mentioned in two preliminary papers published by the writer.[1]

ZYGNEMA COLLINSIANUM nov. sp.

Filamentis caespites laete virides efformans; cellulis vegetativis 18–24 μ latis, 1.5–4-plo longioribus; cellulis fructiferis in medio introrsum inflatis; zygosporis globosis (26–40 μ diam.) vel oblongis (26–40 μ × 30–47 μ), maturitate caeruleis, mesosporio scrobiculis magnis ornato; aplanosporis cylindraceo-oblongis (18–24 μ × 40–76 μ), inter cellulis vegetativis sparsis, caeruleis et scrobiculatis.

Filaments forming bright green masses; vegetative cells 18–24 μ × 32–80 μ, fertile cells inflated on the inner side near the middle; zygospores globose (26–40 μ in diam.) to oblong (26–40 μ × 30–47 μ), blue at maturity, median spore wall marked by large pits; aplanospores cylindrical-oblong, 18–24 μ × 40–76 μ, scattered among the vegetative cells, color and markings similar to the zygospores.

This species is evidently closely related to *Zygnema peliosporum* Wittrock, but differs in the dimensions, form, and markings of the spores. It has been found in McCabe's pond and the railroad ditches between Briscoe and Casey, Illinois. In the material from McCabe's pond (Coll. No. 2271) the spores are formed in either of the conjugating filaments or in the tube between the two. Type in herb. E. N. T. Collections No. 1076, 1077. Plate XXV, figures 1–3.

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[1] Transeau, E. N., The periodicity of Algae in Illinois. Trans. Amer. Microsc. Soc. **32**: 31–40. 1913; Annotated List of the Algae of Eastern Illinois. Trans. Illinois Acad. Sci. **6**: 69–89. 1913.

ZYGNEMA DECUSSATUM (Vauch.) nov. comb.

Synonyms: *Zygnema pectinatum* (Vauch.) Ag. var. *decussatum* (Vauch.) Kirch., *Zygogonium decussatum* (Vauch.) Kuetzing, *Tyndaridea decussata* Hassall, *Conjugata decussata* Vaucher.

Both *Z. pectinatum* (Vauch.) Ag. and the so-called variety *decussatum* (Vauch.) Kirch. are common in the ponds, pools, and ditches of eastern Illinois. They have little in common except that both form scrobiculate spores in the conjugating tube. They sometimes occur in the same ponds but more frequently not. The species in the permanent ponds is essentially a perennial, while the variety in all stations is a late spring annual. *Z. decussatum* forms vivid green flocculi on the surface of ponds in which it occurs. *Z. pectinatum* is usually submerged and possesses a thick mucilaginous outer wall. The cells of the former are 3–5 times as long as the diameter, of the latter from 1–3 times. The dimensions of the former throughout are but little more than half those of the latter. In view of all these differences it seems best to separate the variety and give it specific rank.

SPIROGYRA NARCISSIANA nov. sp.

Filamentis plerumque sparsis; cellulis vegetativis 12–14 μ latis, diametro 15–30-plo longioribus; chromatophoris singulis, gracilis, laxi anfractibus 2–5; dissepimenta cellularum semireplicata; cellulis fructiferis valde medio inflatis (ventricosis vel quasi quadraticis), 25–33 μ latis, haud abbreviatis; aplanosporis ellipticis vel ovoideis, maturitate flavescentibus, 23–30 μ latis, 50–120 μ longis.

Filaments usually scattered; vegetative cells 12–14 μ × 200–400 μ, one chromatophore, slender, making 2–5 turns in the cell; dissepiments semireplicate (i. e. the fold in wall extends only through an arc of 180° instead of 360°, and fold in the wall of the adjoining cell alternates with it); fertile cell much inflated toward the middle, either rounded or quadrate in outline, 25–33 μ in diameter, not shortened; aplanospores elliptical to ovoid, 23–30 μ × 50–120 μ, yellow at maturity.

This species is intermediate between the two divisions of the genus: *Conjugata* (Vauch.) Hansg. and *Salmacis* (Bory.) Hansg. This fact together with its aplanosporic habit of reproduction, and the quadrate form of the inflation of the fertile cells, make it a species of peculiar interest. Thus far it has been recorded from the dam at Urban Park, Charleston, Ill., during September and October, 1912. Type in herb. E. N. T. Collections No. 1682, 1663. Plate XXV, figures 4–8.

Spirogyra tenuissima (Hass.) Kuetz. var. rugosa nov. var.

Cellulis vegetativis 11–13 μ latis; zygosporis 28–32 μ × 55–64 μ, mesosporio subtiliter scrobiculato; ceterum ut in typo. Cum specie passim.

Vegetative cells 11–13 μ in diameter; zygospores 28–32 μ × 55–64 μ, median spore wall minutely pitted, otherwise as in the type. Occurring with the species.

This variety approaches the upper limits of the species in size, and is readily distinguished by its pitted median spore wall. It has been recorded from ponds at Charleston, Casey, and Lerna, a ditch at Dorans, and a small stream southwest of Wrightsville, Illinois. Type in herb. E. N. T. Collections No. 1871, 2270.

Spirogyra inflata (Vauch.) Rab. var. foveolata nov. var.

Cellulis vegetativis et fructiferis ut in typo, zygosporis mesosporio scrobiculato instructis. Cum specie passim.

Vegetative cells and fertile cells as in the type, zygospores with median wall scrobiculate. Occurring with the species.

This variety has been found in the Ice Plant pond, Casey, and in Campus Creek, Charleston, Illinois. It differs from the type only in the very distinct markings of the median spore wall. Type in herb. E. N. T. Collections No. 783, 784.

Spirogyra rectangularis nov. sp.

Filamentis plerumque sparsis; cellulis vegetativis 35–40 μ latis, 4–9-plo longioribus, dissepimenta replicata; chromatophoris 2–4, anfractibus 2–5; cellulis fructiferis medio valde inflatis, quadraticis, 48–70 μ latis; zygosporis ovatis vel cylindraceo-ovoideis, apice rotundatis, 45–65 μ latis, 75–120 μ longis; maturitate fuscescentibus.

Filaments usually scattered; vegetative cells 35–40 μ × 150–320 μ, dissepiments replicate; chromatophores 2–4, making 2–5 turns in the cell; fertile cells quadrately inflated toward the middle, 48–70 μ in diameter; zygospores ovoid to cylindrical-ovoid, with rounded ends, 45–65 μ × 75–120 μ; yellowish-brown at maturity.

This species is related to *S. quadrata* (Hass.) Petit, which occurs in its typical form in an adjoining pond. It differs in its larger dimensions throughout, greater number of chromatophores, and the form of the zygospores. It has been found for several years in the West Tile Factory pond, Charleston, and a pond on the Gray farm southeast of Lerna, Illinois. Fruits during May. Type in herb. E. N. T. Collection No. 788. Plate XXV, figures 9–11.

SPIROGYRA PRATENSIS nov. sp.

Caespites flavo-virides efformans; cellulis vegetativis 17–20 μ latis, 4–12-plo longioribus; chromatophoris singulis vel binis, anfractibus 1–8; generatio zygosporis vel aplanosporis ortis (conjugatio lateralis vel scalaris); cellulis fructiferis inflatis, ad 55 μ latis; cellulis sterilis tum cylindraceis tum inflatis vel bullatis (ad 90 μ latis); sporis ovoideis, ellipticis vel cylindraceo-ellipticis, 24–36 μ latis, 50–70 μ longis, maturitate flavescentibus.

Forming yellowish green masses; vegetative cells 17–20 μ in diameter, 80–240 μ in length, chromatophores one or two in a cell, making from 1–8 turns; reproduction by zygospores and aplanospores, conjugation lateral and scalariform; fertile cells inflated, up to 55; sterile cells cylindrical, inflated, or bullate (diam. up to 90 μ); spores ovoid, elliptical, or cylindrical with rounded ends, 24–36 μ × 50–70 μ, yellow at maturity.

This species combines most of the characteristics of *S. mirabilis* (Hass.) Kuetz. and *S. polymorpha* Kirchner. From the former it differs in its dimensions (almost all living cells measure exactly 20 μ regardless of the habitat), in the constant occurrence of cells in the filaments with two chromatophores, in its regular production of zygospores, and in the tendency of the sterile cells to be inflated. *S. mirabilis* has been collected in this territory and like the European specimens varies considerably in dimensions, the cell diameter up to 27, and it showed no cells with two chromatophores, and lacked the inflated sterile cells. From *S. polymorpha* this species differs in its dimensions, in its regular production of aplanospores, and in the presence of inflated sterile cells. As shown in the figures some of the fertile cells containing aplanospores have developed protuberances as in conjugation, but apparently without reference to adjoining filaments, as they show a definite tendency to alternate in successive cells. Another interesting feature is the occasional occurrence of cells containing aplanospores which have become attached to sterile cells, but the terminal wall of the protuberance has not been dissolved. This species is probably common in Illinois. I have recorded it from all the larger ponds, and Campus creek at Charleston; also from the Brookhart farm pond southwest of Oilfield. Type in herb. E. N. T. Collections No. 1103, 1822. Plate XXV, figures 12–14; Plate XXVI, figures 1–2.

SPIROGYRA CATENAEFORMIS (Hass.) Kuetz. var. PARVULA nov. var.

Filamentis plerumque sparsis; cellulis vegetativis 20–24 μ latis, 2–5-plo longioribus; chromatophoro uno, anfractibus 1–6; cellulis fertilis ad 37 μ latis inflatis; conjugatione plerumque laterali, rarius scalariformia; zygosporis flavescentibus, ellipticis, 20–27 μ latis, 40–60 μ longis.

Filaments usually scattered; vegetative cells 20–24 μ × 50–105 μ, chromatophore one in each cell, making 1–6 turns; fertile cells inflated up to 37 μ; conjugation mostly lateral, sometimes scalariform; zygospores yellow, elliptical, 20–27 μ × 40–60 μ.

This variety is of such common and general occurrence both in association with the type and without it, and intergrading forms are so rare—especially in the matter of the spores—that it seems advisable to give it a name. It differs principally in its smaller dimensions throughout and in the relatively shorter spores. In this vicinity *S. catenaeformis* usually has a cell diameter of 27–30 μ, rarely as low as 24 μ, while the spore diameter varies from 27–33 μ, usually being 30 μ, with a length of from 2–3 times the diameter. I have recorded the variety from ponds at Charleston, Casey, Oilfield, Lawrenceville, and Lerna. Type in Herb. E. N. T. Collection No. 1876. Plate XXVI, figures 3–4.

SPIROGYRA CIRCUMLINEATA nov. sp.

Cellulis vegetativis 40–48 μ latis, diametro 3–6-plo longioribus, dissepimentis planis; chromatophoro uno, gracili, anfractibus 4–8; cellulis fructiferis uno latere (in quo conjugatio sequiter) inflatis, altero rectis; zygosporis ellipticis, 40–50 μ latis, 70–125 μ longis, maturitate fuscescentibus, episporio lineamento uno plus minus longitudinaliter instructo.

Vegetative cells 40–48 μ in diameter, 120–240 μ in length, dissepiments plane; chromatophore one, slender, making 4–8 turns in the cell; fertile cells swollen on the conjugating side only; zygospores elliptical, 40–50 μ × 70–125 μ, yellowish-brown at maturity, the outer spore wall marked by a more or less longitudinal line.

This species in so far as its fertile cells are concerned has much the same appearance as *S. varians* (Hass.) Kuetz., which occurs in the same ponds. From this species it is distinguished by the larger dimensions throughout, by the greater number of turns of the chromatophore, the absence of inflated sterile cells, and the rather prominent line extending around the spore. Recorded only from Marshall pond, four miles north of Charleston, Illinois, and the ponds on the Gray farm southeast of Lerna. Fruits during May and early June. Type in herb. E. N. T. Collection No. 1353. Plate XXVI, figures 5–6.

Spirogyra velata occidentalis nov. var.

Caespites laete virides efformans; cellulis vegetativis 36–53 μ latis, diametro 3–7-plo longioribus; chromatophoris 1–3, anfractibus 2–6; cellulis fructiferis cylindraceis vel inflatis, ad 66 μ latis; zygosporis ovoideis, 36–56 μ latis, mesosporio scrobiculato, maturitate fuscescentibus.

Forming bright green masses; vegetative cells 36–53 μ in diameter, 125–300 μ in length; chromatophores 1, 2, or 3, making 2–6 turns in the cell; fertile cells cylindrical or enlarged up to 66 μ in diam.; zygospores ovoid, 36–56 μ × 57–105 μ, median spore wall scrobiculate; spore color at maturity yellowish brown.

This species has been found in the remnant of an old prairie pond on the Brookhart farm, southwest of Oilfield, and the small stream at Lerna, Illinois. It is closely related to *S. velata* Nordst., from which it differs in the number of chromatophores, larger dimensions of the vegetative cells, and the color of the mature spores. Type in herb. E. N. T. Collection No. 1826. Plate XXVI, figures 8–9.

Spirogyra punctiformis nov. sp.

Filamentis plerumque sparsis; cellulis vegetativis 27–30 μ latis, 4–14-plo longioribus, dissepimentis planis; chromatophoris gracilis 1–2, anfractibus 3–6; cellulis fructiferis inflatis (44–50 μ latis); cellulis femineis 100–250 μ longis, masculis 90–140 μ, singulis vel binis cellulis vegetativis alternis; tubo conjugationis plerumque ex cellula mascula emisso; zygosporis 40–48 μ × 60–110 μ, ovatis vel cylindraceo-ovoideis, mesosporio punctulis obsito, maturitate flavescentibus.

Filaments usually scattered; vegetative cells 27–30 μ × 120–390 μ, end walls plane; chromatophores one or two, narrow, making from 3–6 turns in the cell; fertile cells inflated (44–50 μ in diam.); female cells 100–250 μ long, male cells 90–140 μ long, occurring singly or in pairs, alternating with vegetative cells; conjugating tube usually produced by the male cell; zygospores ovate to cylindrical-ovoid, 40–48 μ × 60–110 μ, median spore wall punctate, yellow when mature.

In some respects this species resembles *S. punctata* Cleve, but differs in the number of chromatophores, in the size and form of the zygospores, and the frequent occurrence of the conjugating cells in pairs. Of considerable interest is the fact that the male cell is always shorter than the female. It fruits from late May to July in the New, and East Tile Factory ponds, Charleston, Illinois. Type in herb. E. N. T. Collections No. 627, 1986. Plate XXVI, figure 7.

Spirogyra ellipsospora nov. sp.

Filamentis saturate viridibus, lubricis; cellulis vegetativis 125–

150 μ latis, diametro 1–3-plo longioribus; chromatophoris 3–8, anfractibus ½–4; zygosporis ellipticis plus minus acuminatis, fuscescentibus, 100–140 μ latis, 160–255 μ longis.

Filaments dark green, lubricous; vegetative cells 125–150 μ × 125–500 μ; chromatophores 3–8, making ½–4 turns in a cell; zygospores elliptical with more or less pointed ends, brownish yellow at maturity, 100–140 μ × 160–255 μ.

The vegetative filaments of this form are quite indistinguishable from those of *S. crassa* Kuetz. which also occurs in this vicinity. The form and dimensions of the spores however are amply distinct. Known from the pond at the western edge of Casey, and from Hodgen's pond, Charleston, Illinois. Fruits during the summer and early autumn. Type in herb. E. N. T. Collections No. 1485, 1406, 1403, 766, 769, 1352. Plate XXVII, figure 1.

Spirogyra ellipsospora var. crassoidea nov. var.

Cellulis vegetativis 140–150 μ latis, diametro 1–4-plo longioribus; zygosporis late ellipticis et secundum positionem apice attenuatis, 120–140 μ latis, 145–255 μ longis; ceterum ut in typo.

Vegetative cells 140–150 μ × 140–560 μ, zygospores compressed elliptical, ends broadly rounded in one position, sharply pointed in another, 120–140 μ × 145–255 μ; otherwise as in the type.

This variety has been recorded only from the Tile Factory ponds, Charleston. The form of the spore distinguishes it from the type, and the fact that the spores are compressed elliptical instead of compressed ovate separates it from *S. crassa* Kuetz. Type in herb. E. N. T. Collection No. 1507. Plate XXVII, figure 2.

Spirogyra submaxima nov. sp.

Filamentis plerumque caespites saturate virides lubricos efficientibus, rarius sparsis; cellulis vegetativis 70–110 μ latis, diametro 1½–4-plo longioribus; chromatophoris 8–9, anfractibus .1–1; cellulis fructiferis tum modice tumidis tum cylindraceis; zygosporis lentiformibus, maturitate bruneis, crass. 50–75 μ, diam. 70–100 μ, mesosporio levi.

Filaments usually forming dark green lubricous masses, more rarely scattered; vegetative cells 70–110 μ in diameter, 100–300 μ long; chromatophores 8–9, making 1/10 to 1 turn in a cell; fertile cells slightly inflated or cylindrical; sterile cells not enlarged; zygospores lenticular, brown, 50–75 μ in thickness, 70–110 μ in diameter; median spore wall smooth.

This species has commonly a very thick pectose sheath, occasionally as thick as 17μ. It is distinguished from *S. maxima* (Hass.) Wittr. by the greater number of chromatophores, by the longer cells as compared with the width, by the smaller dimensions throughout, and the smooth median wells of the zygospores. From *S. majuscula* Kuetz. it is readily separated by the larger dimensions, number of chromatophores, and usually the greater curvature of the chromatophores. During some seasons this alga begins to fruit in May and continues to fruit until September. In other years spore formation extends from July to November. Thus far it has been recorded only from the East Big Four pond, three miles east of Charleston, and a "cut off" from Polecat creek, south of Ashmore. Type in herb. E. N. T. Collections No. 233, 246; 1461, 1647. Plate XXVII, figures 3–4.

SPIROGYRA ILLINOIENSIS nov. sp.

Filamentis in caespites sordide virides et intricatos consociatis; cellulis vegetativis 65–85 μ latis, diametro 1.5–4-plo longioribus; chromatophoris 6–9, angustis, modo subrectis longitudinalibus, modo spiralibus, anfractibus .1–1; cellulis conjugatis abbreviatis, paulo inflatis et geniculatis, canalis conjugationis brevis et latis; cellulis masculis brevioribus quam femineis; zygosporis 85–115 μ diam., 140–190 μ longis, ovato-ellipticis vel ellipticis, mesosporio crasso et punctato, maturitate flavescentibus.

Filaments forming dull green tangled masses; vegetative cells 65–85 μ × 100–300 μ; chromatophores 6–9, narrow, nearly straight and longitudinal, or spiral, making from .1–1 turn in the cell; conjugating cells shortened, somewhat inflated and geniculate, conjugating tube short and broad; male cell shorter than the female; zygospores 85–115 μ × 140–190 μ, ovoid-elliptical to elliptical in form, median spore wall thick and punctate, yellow when mature.

Evidently related to *S. stictica* (Eng. Bot.) Wille, from which it is distinguished by the larger dimensions throughout, greater number of chromatophores, and the punctate walled spores. From *S. ceylanica* Wittr. it differs in the number of chromatophores, and the markings of the spore wall. Conjugation is initiated by the bending of the gametangia and the development of slight prominences on both cells. This is followed by a mucilaginous secretion at the point of contact, which may persist as a ring about the tube for some days after the union of the cells is complete. After contact the chromatophores of the gametangia become greatly enlarged and engorged with starch and fatty bodies, enlargement of the cells continues, but stops in the case of the male cell when the male gamete passes over.

This species has been found only in the pond on the Gray farm,

southeast of Lerna, Illinois. It fruits during May. Type in herb. E. N. T. Collections No. 1377, 1374, and 1842. Plate XXVIII, figures 1–3.

MOUGEOTIA TUMIDULA nov. sp.

Cellulis vegetativis 6–8.5 μ latis, 10–20-plo longioribus; zygosporis inter 4 cellulas sitis, tumidis quadrangularibus 22–26 μ × 26–30 μ, a latere visis ellipticis, angulis retusis, mesosporio hyalino evidenter subtiliter scrobiculato.

Vegetative cells 6–8.5 μ × 70–120 μ, zygospores adjoined by four cells; quadrate, somewhat tumid, 22–26 μ × 26–30 μ, elliptical when seen from the side, angles retuse, median spore wall distinctly but minutely scrobiculate.

This species resembles in size and retuse corners of the zygospore *Mougeotia viridis* (Kuetz.) Wittr. but differs in having the spore walls convex instead of concave, scrobiculate instead of smooth. It differs from *M. gracillima* (Hass.) Wittr. in its larger size, retuse angled spores, and the convex spore walls. Type in herb. E. N. T. Collection No. 744. Found thus far only in the Embarras river, Wheeler, Illinois, Sept. 1911. Plate XXVIII, figure 4. (Scale of figure 1 cm. = 25 μ.)

OEDOGONIUM PRATENSE nov. sp.

Oedogonium dioicum, macrandrium, oogoniis singulis, rarissime binis, subdepresso-globosis vel late pyriformi-globosis, opercula apertis, circumscissione mediana, angusta sed distincta; oosporis depresso-globosis vel subglobosis, partem oogoniorum inflatum complentibus, membrana laevi; plantis masculis paulo gracilioribus quam femineis; antheridiis 1–2-cellularibus, saepe cum cellulis vegetativis alternis; spermatozoidis singulis; cellula fili basali forma, ut vulgo elongata;

crassit. cell. veget. plant. fem.	10–17 μ,	altit. 35–95 μ;
crassit. cell. veget. plant. masc.	8–15 μ,	altit. 30–70 μ;
crassit. oogon.	33–40 μ,	altit. 33–50 μ;
crassit. oospor.	32–38 μ,	altit. 28–35 μ;
crassit. cell. antherid.	10–14 μ,	altit. 13–18 μ.

Dioecious, macrandrous, oogonia single, very rarely two, subdepressed globose or broadly pyriform-globose, operculate, division median, narrow but distinct; oospore depressed globose or subglobose. filling or nearly filling the inflated portion of the oogonium, membrane smooth; male plants more slender than the female; antheridia 1–2-celled, usually alternating with vegetative cells; sperms single; basal cell of filament commonly elongated;

diam. veg. cell, female plant	10–17 μ,	length 35–95 μ;
diam. veg. cell, male plant	8–15 μ,	length 30–70 μ;
diam. oogonia	33–40 μ,	length 33–50 μ;
diam. oospores	32–38 μ,	length 28–35 μ;
diam. antheridial cells	10–14 μ,	length 13–18 μ.

The forms nearest to this species are *Oe. acmandrium* Elfv. and *Oe. psaegmatosporum* Norsdt. From these it is distinguished by its larger dimensions and dioecious habit. Among the poriferous species it bears some resemblance to *Oe. rufescens* Wittr. This species has been collected in Anderson's pools, on the Big Four R. R. right of way, just east of Charleston, Illinois, and in the ponds southeast of Lerna. It fruits in May. Type in herb. E. N. T. Collection No. 1797. Plate XXIX, figures 9–12.

OEDOGONIUM PRATICOLUM nov. sp.

Oedogonium dioicum, (?) nannandrium, idioandrosporum; oogoniis singulis vel rarius 2–7-continuis, ellipsoideis vel globoso-ellipsoideis, saepe terminalis, rarius sparsis; membrana oogonii interdum subcrassa, operculo apertis, circumscissione suprema, operculo minimo, deciduo; oosporis eadem forma ac oogoniis, haec plane complentibus, membrana laevi; cellulis suffultoriis eadem forma ac cellulis ceteris; plantis masculis paululo gracilioribus quam femineis; androsporangiis 1–20-cellularibus; cellulis vegetativis leviter capitellatis; cellula fili basali forma, ut vulgo, elongata; cellula terminali apice apiculata vel in setam longam, tenuem, hyalinam, producta;

crassit. cell. veget. plant. fem	16–26 μ,	altit. 75–100 μ;
crassit. cell. veget. plant. masc	14–22 μ,	altit. 60–100 μ;
crassit oogon	48–60 μ,	altit. 62– 74 μ;
crassit. oospor	46–58 μ,	altit. 60– 72 μ;
crassit. cell. androsp	20–24 μ,	altit. 18– 22 μ.

Dioecious, (?) nannandrous, idioandrosporous; oogonia single or more rarely in groups of 2–7, ellipsoid to globose-ellipsoid, often terminal, sometimes scattered; oogonium wall sometimes rather thick, operculate, division at the upper extremity of the oogonium, lid very small, deciduous; oospores of the same form as the oogonia, which they completely fill, wall smooth; suffultory cells similar to the other vegetative cells; male filaments a little smaller than the female; androsporangia 1–20-celled; vegetative cells slightly capitellate; basal cell of filament usually elongate; terminal cell apiculate or extended into a long, hyaline, tenuous seta;

diam. veg. cells, female plant	16–26 μ,	length 75–100 μ;
diam. veg. cells, male plant	14–22 μ,	length 60–100 μ;

diam. oogonia	48–60 μ,	length 62– 74 μ;
diam. oospores	46–58 μ,	length 60– 72 μ;
diam. androsporangial cells	20–24 μ,	length 18– 22 μ.

This species is closely related to *Oe. obtruncatum* Wittr. It differs in being idioandrosporous, having generally larger dimensions, and in the form of the terminal cell. It has been found in Hodgen's pond, and the East Big Four pond, Charleston, Illinois. Fruits in summer and autumn. Type in herb. E. N. T. Collections No. 1967, and 1963. Plate XXIX, figures 1–5.

OEDOGONIUM ILLINOIENSE nov. sp.

Oedogonium dioicum, nannandrium, gynandrosporum; oogoniis singulis, subglobosis vel oboviformi-globosis, poro mediano apertis; oosporis globosis vel subglobosis, oogonia fere complentibus, membrana duplici; episporio costis spiraliter dispositis, costis spiralibus numero 4–7, utrinque in polo, in sectione horizontali, fere mediano, nunquam verticali sito conniventibus, endosporio laevi; cellulis suffultoriis tumidis; androsporangiis 1–5-cellularibus; cellula fili basali forma, ut vulgo, elongata; nannandribus palululum curvatis, in cellulis suffultoriis sedentibus, antheridio exteriore, 1–4-cellulari;

crassit. cell. veget	13–18 μ,	altit. 76–129 μ;
crassit. cell suffult	32–40 μ,	altit. 50– 73 μ;
crassit. oogon	51–60 μ,	altit. 60– 70 μ;
crassit. oospor	45–56 μ,	altit. 48– 66 μ;
crassit. cell. androsp	13–17 μ,	altit. 17– 22 μ;
crassit. stip. nannandr	14–17 μ,	altit. 37– 57 μ;
crassit. cell. antherid	9–12 μ,	altit. 15– 23 μ.

Dioecious, nannandrous, gynandrosporous; oogonia sugblobose to oboviform-globose, occurring singly in the filaments; pore median; oospores globose or subglobose, nearly filling the oogonia, membrane double; the outer spore wall marked by 4–7 spiral ribs uniting at the poles, the polar axis always placed in a transverse position, never parallel with the filament, the inner spore wall smooth; suffultory cells swollen; androsporangia 1–5-celled; basal cell of the filament elongated; dwarf males slightly curved resting on the suffultory cell, antheridium exterior, 1–4-celled;

diam. vegetative cells	13–18 μ,	length 76–120 μ;
diam. suffultory cells	32–40 μ,	length 50– 73 μ;
diam. oogonia	51–60 μ,	length 60– 70 μ;
diam. oospores	45–56 μ,	length 48– 66 μ;
diam. androsporangia	13–17 μ,	length 17– 22 μ;
diam. dwarf male stipe	14–17 μ,	length 37– 57 μ;
diam. antheridia	9–12 μ,	length 15– 23 μ.

Belongs near *Oe. spirale* Hirn, from which it differs in being gynandrous, in having narrower and longer vegetative cells, swollen suffultory cells. Occurs in the Gray ponds southeast of Lerna, Illinois. Type in herb. E. N. T. Collections No. 1361, 1364. Plate XXIX, figures 6–8.

OEDOGONIUM PAUCO-COSTATUM nov. sp.

Oedogonium dioicum, macrandrium; oogoniis singulis, ellipsoideis, opercula apertis, circumscissione superiore; oosporis ellipsoideis, oogonia fere complentibus, membrana triplici: episporio, in latere exteriore, laevi, mesosporio longitudinaliter costato, costis integris, interdum anastomosantibus, in medio oosporae c : a 15–19; antheridiis 2–8-cellularibus; spermatozoidis binis, divisione horizontali ortis; cellula fili terminali obtusa, cellula basali forma, ut vulgo, elongata;

crassit. cell. veget. plant. fem.... (15–)	20–27 μ,	altit. 70–155 μ;
crassit. cell. veget. plant. masc.... (15–)	19–25 μ,	altit. 70–160 μ;
crassit. oogon.	54–60 μ,	altit. 70–104 μ;
crassit. oospor.	50–56 μ,	altit. 66– 90 μ;
crassit. cell. antherid.	18–23 μ,	altit. 8– 12 μ.

Dioecious, macrandrous, oogonia single, ellipsoid, operculate, division superior; oospore elliptical nearly filling the oogonium, membrane triple: outer wall smooth on the outer side, median wall longitudinally ribbed, ribs from 15–19 in number, inner wall smooth; antheridia 2–8-celled; sperms two, division horizontal; terminal cell obtuse, basal cell usually elongate;

diam. veg. cells, female fil...... (15–)	20–27 μ,	length 70–155 μ;
diam. veg. cells, male fil........ (15–)	19–25 μ,	length 70–160 μ;
diam. oogonia	54–60 μ,	length 70–104 μ;
diam. oospores	50–56 μ,	length 66– 90 μ;
diam. antheridial cells	18–23 μ,	length 8– 12 μ.

This species is near *Oedogonium Australicum* Hirn, but differs in being larger in most dimensions, in having elliptical instead of globose-elliptical spores, and in having a smaller number of ribs on the median spore wall. It has been recorded only from the Ice Plant pond, Casey, Illinois, where it was associated with *Oe. praticolum* (described above) and *Oe. taphrosporum* Nordst. and Hirn. during July, 1912. Type in herb. E. N. T. Collection No. 1495. Plate XXVIII, figure 5.

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EXPLANATION OF PLATES XXV–XXIX

PLATE XXV

FIG. 1. Fertile cells of *Zygnema Collinsianum* showing variable position of zygospore in one or the other filament, or in the tube.

FIG. 2. Mature zygospores of *Zygnema Collinsianum.*

FIG. 3. Mature aplanospore and rhizoid of *Zygnema Collinsianum.*

FIGS. 4–8. Vegetative cells and aplanospores of *Spirogyra Narcissiana.*

FIGS. 9–11. *Spirogyra rectangularis.*

FIGS. 12–13. Spirogyra pratensis forming aplanospores.

FIG. 14. Lateral conjugation in *Spirogyra pratensis.*

PLATE XXVI

FIG. 1. Scalariform conjugation in *Spirogyra pratensis.*

FIG. 2. Usual form of vegetative cells of *Spirogyra pratensis.*

FIGS. 3–4. *Spirogyra catenaeformis* var. *parvula.*

FIGS. 5–6. *Spirogyra circumlineata.*

FIG. 7. *Spirogyra punctiformis.*

FIGS. 8–9. *Spirogyra velata* var. *occidentalis.*

PLATE XXVII

FIG. 1. *Spirogyra ellipsospora.*

FIG. 2. Two views of spore of *Spirogyra ellipsospora* var. *crassoidea.*

FIG. 3. Vegetative cell of *Spirogyra submaxima* showing mucilaginous sheath.

FIG. 4. Zygospores of *Spirogyra submaxima.*

PLATE XXVIII

FIGS. 1–3. Stages in conjugation of *Spirogyra illinoiensis.*

FIG. 4. Mature spore of *Mougeotia tumidula.* This figure is drawn to a larger scale than the others, 10 mm. = 25 μ.

FIG. 5. *Oedogonium pauco-costatum.*

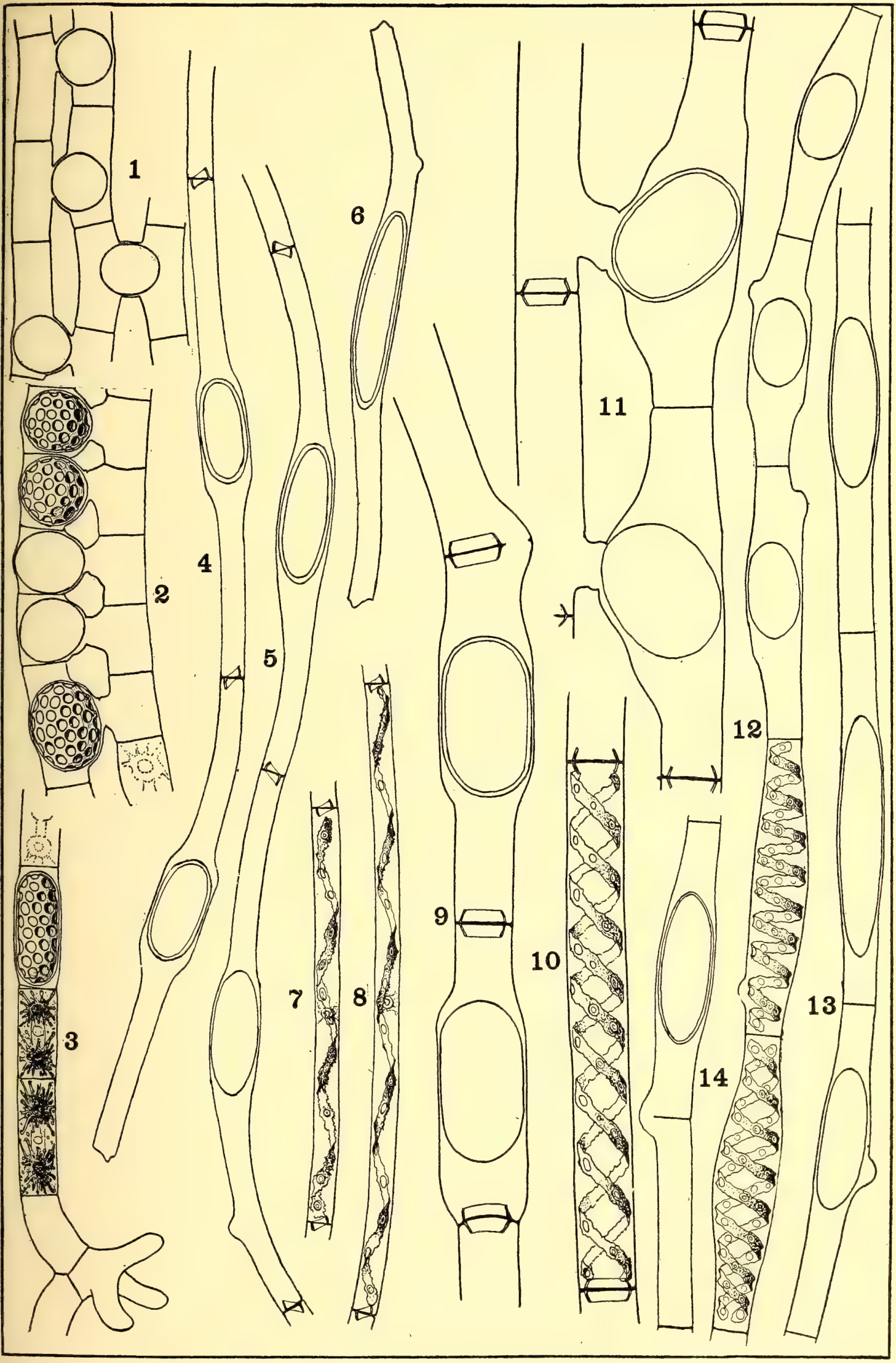
PLATE XXIX

FIGS. 1–5. *Oedogonium praticolum.*

FIGS. 6–8. *Oedogonium illinoiense.*

FIGS. 9–12. *Oedogonium pratense.*

Scale of all drawings, with exception of one noted above, 10 mm. = 45 μ.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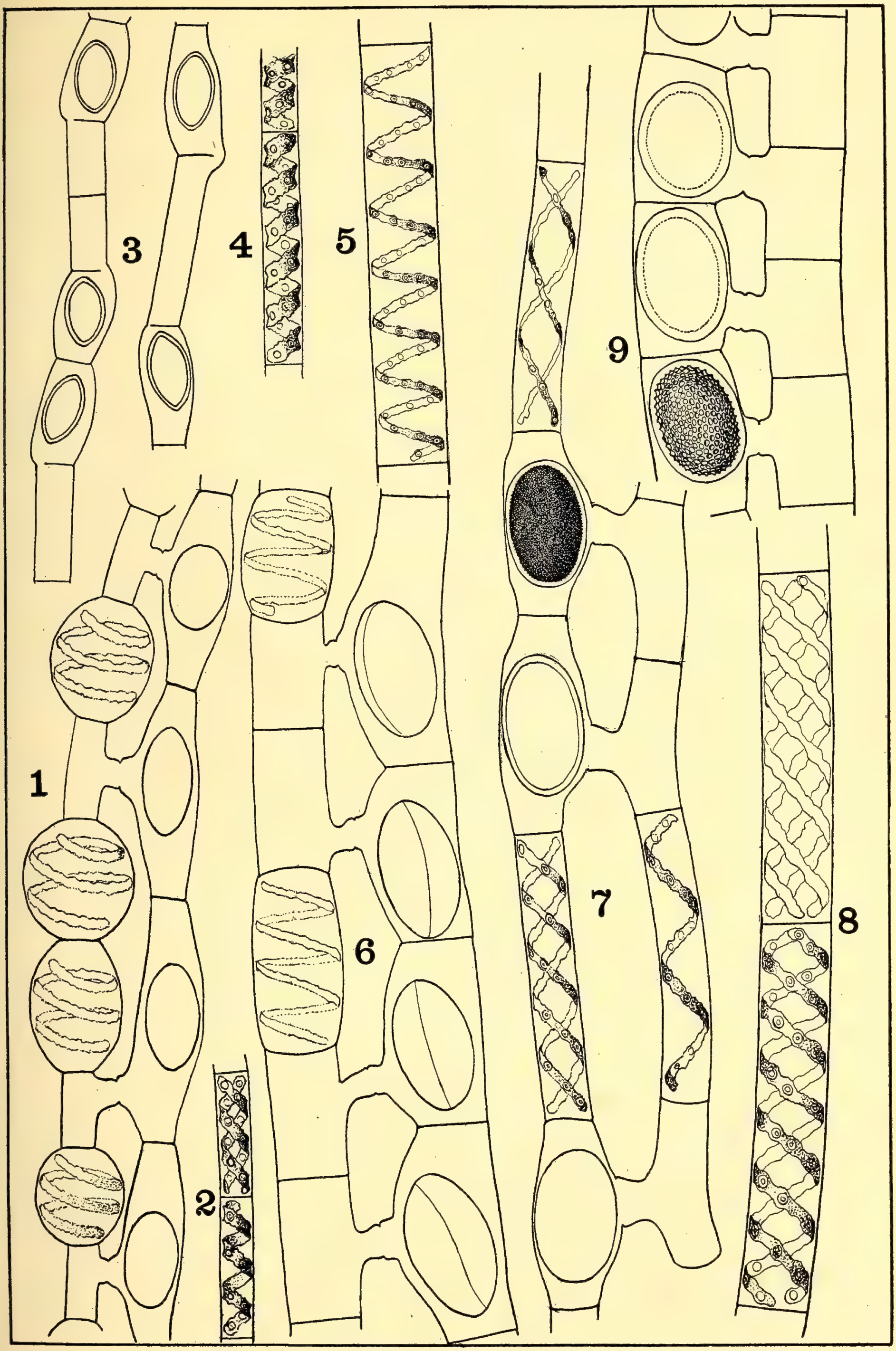
TRANSEAU: NEW SPECIES OF GREEN ALGÆ.



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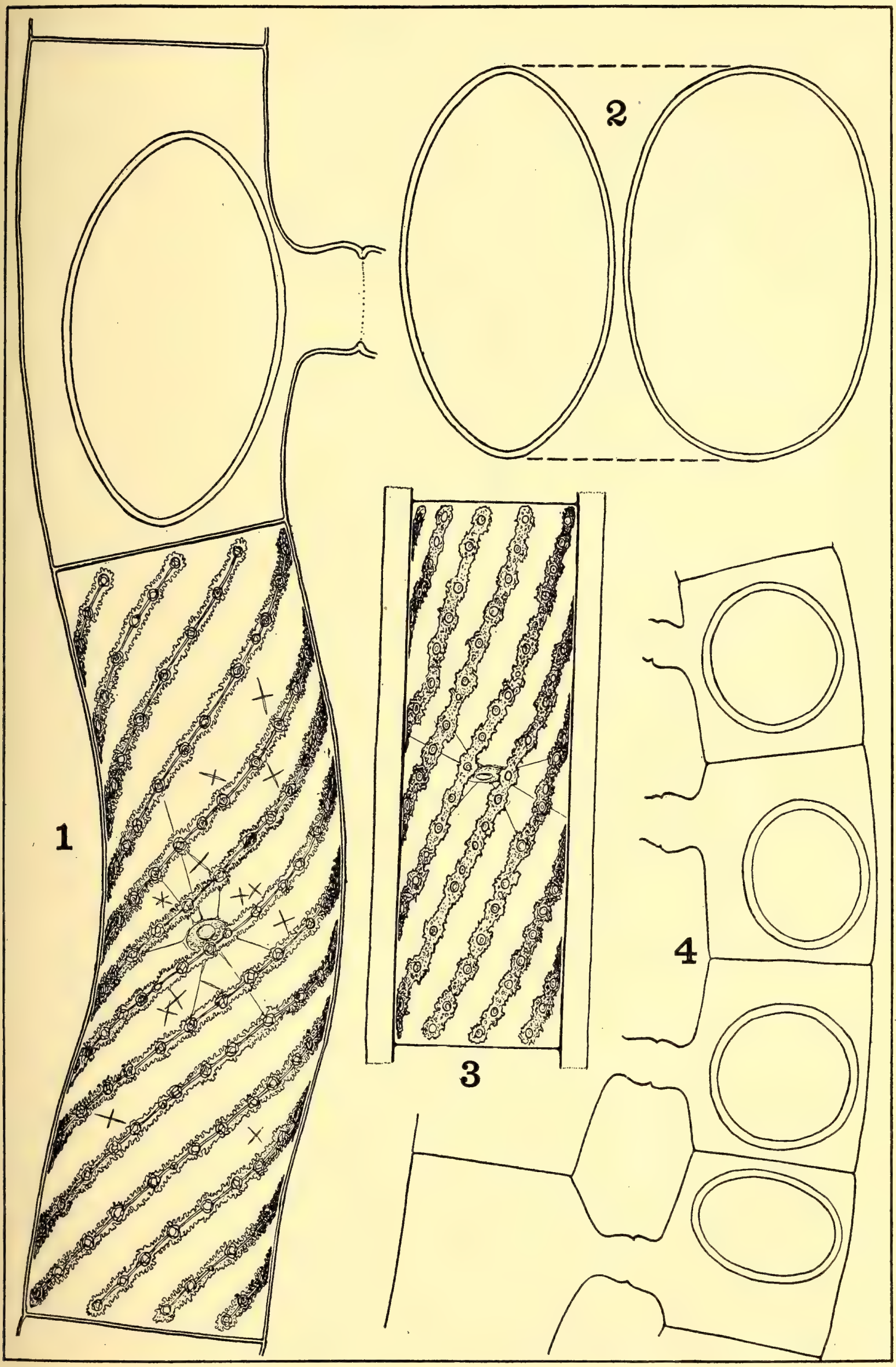

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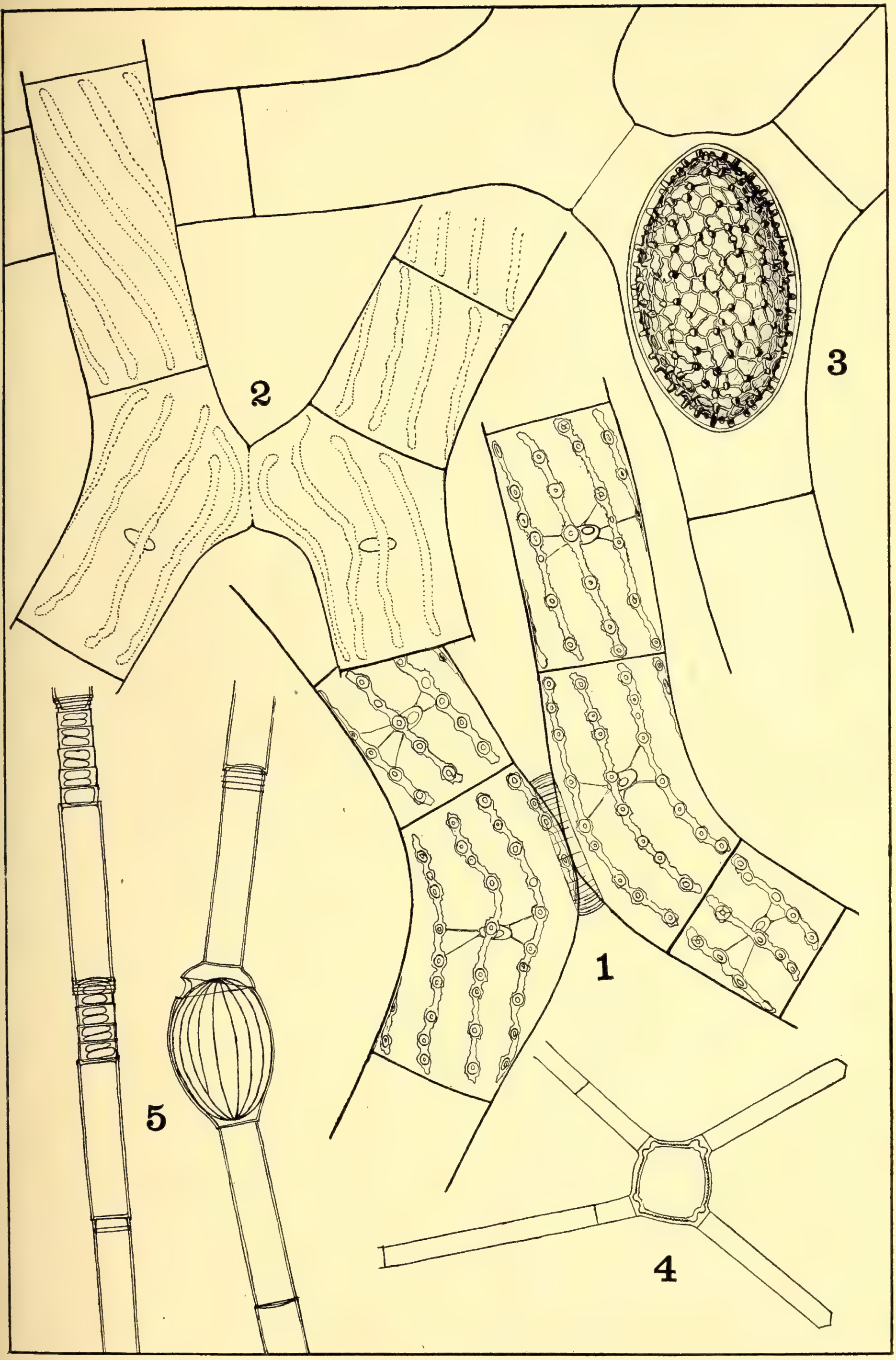
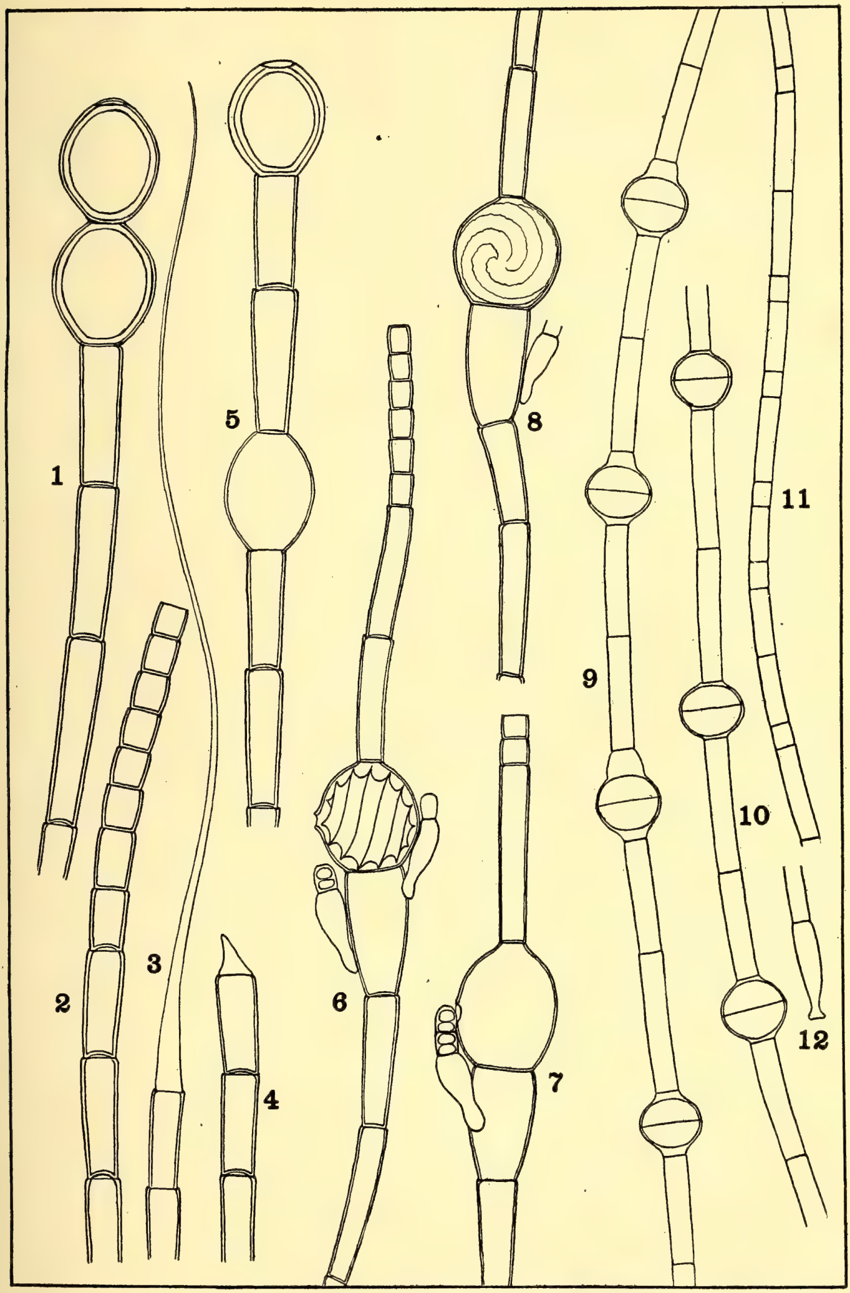


Transeau: New species of green algæ.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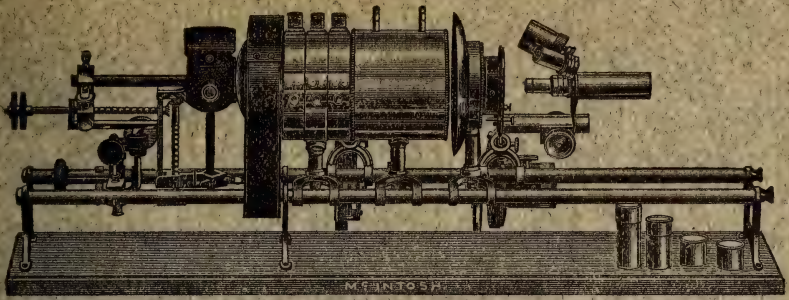

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Marchantia, archegonia
Marchantia, antheridia
Anthoceros, sporophyte
Fern, sorus with sporangia
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Pine stem, cross section
Pine stem, longitudinal section
Equisetum, section of sporophyll
Lily, anther with pollen
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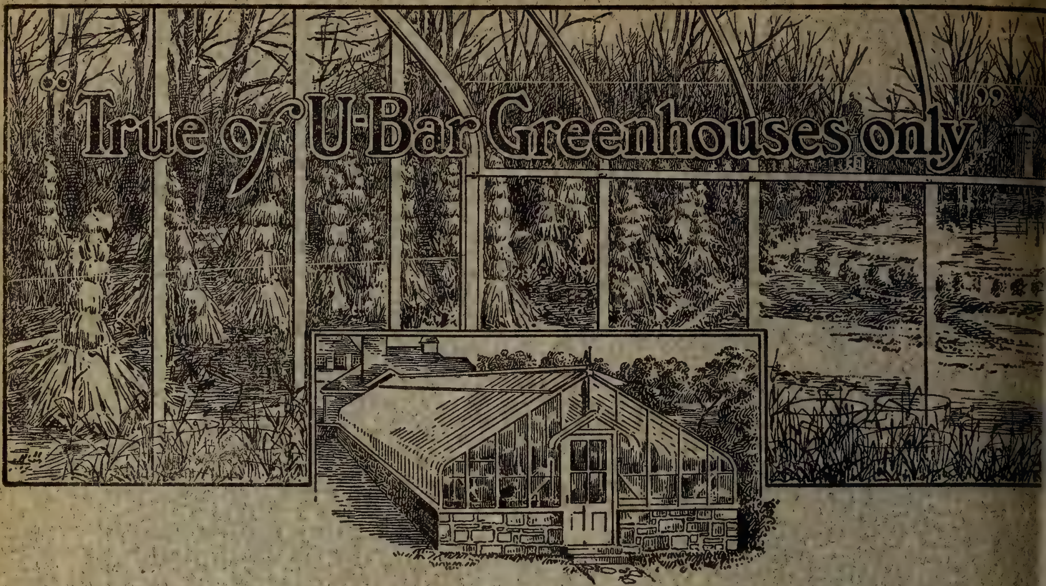
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Vol. I JULY, 1914 No. 7

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INVESTIGATIONS ON THE PHYLOGENY OF THE ANGIOSPERMS

I. THE ANATOMY OF THE NODE AS AN AID IN THE CLASSIFICATION OF ANGIOSPERMS

EDMUND W. SINNOTT

It is generally recognized that during the course of evolution among vascular plants certain rather definite organs or regions of the body have changed much more slowly than others and hence retain many ancient characters which have been lost elsewhere. One of the most important tasks before the student of comparative plant morphology is to determine where these regions are and in what features they are conservative, and thus to aid the phylogenist in picking out those primitive and constant characters on which he may construct a natural system of classification.

The reproductive organs, root, young plant, first annual ring, leaf and node in various families have all been shown to be regions which in a greater or less degree are apt to be conservative in their internal or external structure. Among these the anatomy of the leaf, particularly at the node where leaf and stem unite, often retains in a most striking way features which have been lost elsewhere in the plant. The ancient centripetal or "cryptogamic" wood has persisted in the foliar bundle of Equisetum (3), the cycads (5), and Prepinus (4) after it has disappeared in all other regions, save occasionally in the reproductive axis. The presumably primitive number and arrangement of leaf bundles persists at their point of insertion at the node in the sigillarians, ferns (6), cycads, Cordaitales, Ginkgo and the broad-leaved conifers although it has changed greatly in the petiole and blade.

Since the vascular system of the leaf in the lower orders appears so generally to be a region which is slow to change, it is reasonable to suppose that in the angiosperms as well it will display a similar

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conservatism. The structure of the angiospermous petiole, indeed, has been the subject of numerous and careful studies by different anatomists, and it has been found that in a few families and even in certain genera the petiolar structure is sufficiently peculiar and conservative to be used as a diagnostic character for the group. In general, however, the number and arrangement of the petiolar bundles is too much dependent on the size and texture of the leaf to be of very great taxonomic importance; and even where these characters are reasonably constant, it is very hard to draw phylogenetic conclusions from them and to determine which particular type is the most primitive. The fact that the node of several of the lower orders, rather than the petiole, seems to be a notably conservative region suggested that in the higher seed plants, as well, it might be worthy of study. The present investigation has therefore had as its object an examination of the nodal structures of the angiosperms with the hope of discovering simple and constant anatomical features which will be of value in determining broad lines of relationship.

In the Lycopodiales, ancient and modern, the vascular supply for the leaf is at its base almost invariably a single strand. In the ferns, cycads, Cycadofilices, Cordaitales, Ginkgoales and conifers the foliar bundle, although often single, seems primitively to have been a double one. This double trace, especially in the ferns, has often been broken up into a wide arc of strands. In these lower vascular plants the foliar supply, whether single, double or multiple, causes typically but a single gap (if any) in the continuity of the vascular ring (*fig.* 1). Thomson (7) has observed, however, that in very vigorous specimens of Agathis the two bundles of the leaf-trace are separated at their insertion by a tiny segment of the secondary wood and thus cause a double gap in the cylinder (*fig.* 2). Among the Gnetales the genus Ephedra, which most closely approaches the conifers in other respects, has like them a double leaf-trace, but the two portions as in Agathis are separate at their insertion (*fig.* 3). The node of Gnetum is much more complex for here there are from seven to eleven strands passing off into each leaf and every strand causes a gap of its own in the cylinder.

Such a condition where there is an odd number of bundles, each departing from a distinct gap in the vascular ring, is typical of the Angiosperm node, and has apparently arisen as an adaptation to the increased transpiration current passing to the broad leaves. The particular number of bundles and gaps is extremely constant among related forms, and almost every family has a characteristic nodal formula which is subject to little variation.

As to what was the primitive structure of the node among angiosperms we cannot be sure, but in all probability the earliest condition was rather plastic and variable. It seems clear, however, that a foliar supply of three bundles, each causing a gap of its own in the stem cylinder, is certainly a very ancient type among the dicotyledons. We may designate this as the *trilacunar* condition (*figs.* 4, 5, 11, etc.). It characterizes most of the members of the Piperales, Salicales, Myricales, Juglandales, and Fagales—in short, of the former Amentiferae; and is present in the great majority of the Ranales and Rosales, as well. Since in all likelihood one of these great groups approaches the primitive angiosperms (or at any rate the primitive dicotyledons) in its character, we may feel reasonably sure that the trilacunar condition became fixed in the angiosperm line very far back. This is rendered still more probable by the fact that all other variations of nodal structure in the phylum seem to have been derived, as we shall see, from such a three-bundled type. This type is not confined to the lower Archichlamydeae but also characterizes in general the Rhoedales, Geraniales, Rhamnales, Malvales and many Sapindales and Parietales among the Archichlamydeae; and the Plantaginales, Cucurbitales, Caprifoliaceae and Compositae among the Metachlamydeae, thus occurring in the majority of dicotyledons.

This ancient trilacunar condition has been modified both by reduction and by amplification. In many orders we find that only a single gap is left by the foliar supply, whether the latter is single or multiple (*figs.* 14, 16, 20 etc.). Such a single-gapped or *unilacunar* type of nodal structure characterizes all the Centrospermae, most of the Myrtiflorae and numerous families of the Sapindales and Parietales among the Archichlamydeae; and the Ericales, Primulales, Ebenales, Contortae, Tubiflorae and families in the Rubiales and Campanulatae among the Metachlamydeae. That this simple condition is not a primitive one, however, but has been derived by reduction from the trilacunar type is indicated by a study of the node in those few families which are transitional from one type to the other.

The Cruciferae are perhaps the best example of such a family. Most of the genera in the Thelypodieae, Sisymbriineae, Cochleariineae and Brassicineae, as well as scattering genera in other tribes, possess a foliar trace of three rather distant strands, each causing a gap of its own in the stele. In certain genera and species, however, such as Sisymbrium leiocarpum (*fig.* 13), these three bundles approach each other very closely and are separated only by two very small segments of the cylinder. In all the other Cruciferae the departure of the foliar supply causes but a single gap in the wall of the cylinder, but in such cases the leaf trace itself almost always consists of three bundles (*fig.* 14). In the Cruciferae the three originally distant strands thus seem to have gradually become approximated until by the disappearance of the separating segments of the stele, they come off close together and cause but a single gap. In the Dilleniaceae a very similar transition is also evident.

In many of the groups of plants which are characterized by a unilacunar nodal structure the leaf-trace at its origin frequently consists, as in the Cruciferae, of three distinct strands or of a deeply three-lobed one (*photo.* 3). Such a structure is particularly common in those great unilacunar orders the Centrospermae and the Tubiflorae and indicates that the nodal condition in these and similar groups has arisen by reduction from the primitive three-bundled type.

In certain instances even this triple division of the leaf-trace has been lost in the older parts of the plant, as is shown by *figure* 8, which is a section through the mature stem of *Chenopodium album.* In the young plant, however, where the whole stem structure is simpler, the leaf-trace consists of three quite distinct strands (*fig.* 7). Such "recapitulations" are of frequent occurrence.

Among such families as the Amaranthaceae which are characterized by anomalous growth of the vascular tissue, the leaf-trace is often exceedingly complicated at maturity (*fig.* 10). In the simpler members of the family, however (*fig.* 9), and in the seedlings of the complex forms, the primitive triple condition is retained. The ancestors of such plants probably possessed the nodal structure of primitive angiosperms, with three isolated bundles departing to the leaf. These first became approximated and the gaps fused; and later the foliar supply underwent various further modifications.

The unilacunar condition seems to have been derived from the trilacunar, in certain families, by the abortion of the two lateral strands instead of their fusion with the central trace. In *Ilex opaca* (*fig.* 19), for example, the three typical bundles are usually present, but one or both of the lateral ones tend to be very small. In all other members of this and other genera of the family, however, only a single bundle was observed (*fig.* 20). This shows no signs of being divided into three parts, and without doubt represents the median one of the original three strands. In various species of Myrica the two lateral bundles are usually small and have often disappeared. Spiraea, Exochorda and their immediate allies alone among the Rosaceae, as far as the writer has observed, possess but a single unlobed and undivided leaf-trace (*fig.* 16). In such closely related forms as Physocarpus, however, there are the three widely separated strands (*fig.* 15) and we are forced to the conclusion that the trace of Spiraea represents only the median one of these. This method of reduction seems to have been a common one. From the fact that the single bundle given off to the leaf in the Ebenales, Ericales, Contortae and certain other groups is continuous and undivided we may infer that the unilacunar condition in these forms has arisen by the loss of the two lateral traces rather than by the fusion of all three, as in the Centrospermae and Tubiflorae.

In these two ways, then, the presumably primitive trilacunar condition of the node has been reduced. In several groups, however, the opposite has happened and amplification has taken place. This tendency is shown in many forms but reaches its highest development in the Polygonales and Umbelliflorae. In these orders the leaves are typically sheathing and are supplied by a large number of bundles, each of which causes a gap of its own in the cylinder (*fig.* 6). In certain of the Polygonales, however, such as the dioecious species of Rumex (*fig.* 5), and the climbing forms of Polygonum, the node is trilacunar. In the Araliaceae, also, which are presumably more primitive than the Umbelliferae, the number of bundles and gaps is smaller than in the latter family, being as low as five in certain species. In young plants of the Polygonales and Umbelliflorae the first formed leaves are almost always supplied with three strands which are inserted separately on the vascular ring. In the Ranunculaceae, too, the more primitive genera have three bundles and gaps, and the more highly specialized ones, many. It therefore seems very probable that this *multilacunar* condition has been derived by the amplification of our hypothetically primitive trilacunar type.

If this supposition is correct, it furnishes evidence of much value in determining the relative antiquity of monocotyledons and dicotyledons. The typically sheathing leaf of the former group is supplied by a large number of bundles which come off around the entire periphery of the stem, much as in the multilacunar dicotyledons save that, of course, a continuous vascular ring with typical leaf gaps is absent. In families which are admitted to be very primitive, however, such as the Potamogetonaceae, a much simpler condition prevails. The anatomy of various members of this family has been investigated by Chrysler (2), who comes to the conclusion that the genus Potamogeton is the most primitive in the group; and that its more robust species, such as *P. pulcher*, represent the original type from which the others have been reduced. The vascular tissue in this form is arranged in a cylinder, as in the dicotyledons. The foliar traces are three in number. After running for a short distance in the pith, they leave the cylinder from three distinct points (*fig. 29*), thus presenting a striking resemblance to the trilacunar type which we have regarded as primitive for the dicotyledons, and suggesting the way in which the more complicated nodal structure of the higher monocotyledons has arisen.

The young plants of many of these higher types show decided anatomical resemblances to the primitive monocotyledons and to the dicotyledons. Chrysler (1) has investigated the anatomy of the seedling in several groups, particularly the Araceae and Liliaceae. From his description and figures it is evident that in a great many instances the first few leaves are supplied with three bundles, each of which arises separately and causes a gap of its own in the cylinder which in these earlier stages much resembles the woody ring of dicotyledons. *Figure* 30 shows the node of a seedling of *Acorus calamus* with the three traces departing to one of the earlier leaves.

If such forms as the Potamogetonaceae are actually primitive among monocotyledons, and if the structure of the young plant is rightly regarded as displaying primitive characters—conclusions for both of which there seems to be ample evidence—then it would certainly appear that the monocotyledons have had their origin from plants possessing a medullated ring of vascular tissue and the trilacunar nodal condition which we have regarded as primitive for dicotyledons.

We have already remarked that almost every family of the dicotyledons has a particular type of nodal structure which is extremely constant. That it is almost entirely independent of the size, shape or mode of attachment of the leaf is also apparent. The large pinnate leaves of Fraxinus, Juglans and Daucus, which are essentially similar in size and shape, are provided, respectively, with one, three and many traces, each of which arises independently from the vascular cylinder.

The vascular supply to the large leaves of Catalpa comes off from a single gap, whereas that to the much reduced ones of certain of the Polygonaceae is made up of a considerable number of separately inserted strands. The sheathing leaves of the Polygonaceae and Umbelliferae are supplied by a large number of bundles, but in many instances in the Caryophyllaceae and other groups the leaves also encircle the stem with their bases but into each passes only a single strand from the stem. To be sure, there are a considerable number of instances where the leaf has been so much reduced from adaptation to xerophytic or hydrophytic conditions that it is supplied by a single bundle instead of by the three or more which may distinguish the rest of its family. In such cases, which of course are comparatively rare, the anatomy of the node is of little value in classification. The type for a family should be obtained from vigorous, unreduced species.

Let us now briefly compare the anatomy of the node in the various main groups of dicotyledons and endeavor to find what information a study of this region presents which will be of value in determining relationships.

VERTICILLATAE[1]

Each of the much reduced, scale-like leaves of Casuarina is supplied with a single small trace.

PIPERALES

Among the Piperaceae the genus Peperomia has a normal vascular structure and a trilacunar trace. In Piper, where the stem anatomy is usually anomalous, the foliar supply consists generally of five or seven separate strands. Peperomia thus presumably represents the primitive condition for the family.

SALICALES

In all species of Salix and Populus examined, the nodal structure was invariably trilacunar.

GARRYALES

The node of Garrya is trilacunar.

MYRICALES

The node in this group is doubtless primitively trilacunar, but the two lateral bundles are small and often absent.

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[1] The nomenclature and classification is that of the last (7th) edition of Engler's "Syllabus der Pflanzenfamilien."

LEITNERIALES

Leitneria is trilacunar.

JUGLANDALES.

The order is prevailingly trilacunar, although five bundles and gaps frequently occur in Pterocarya.

JULIANALES

This order is trilacunar.

FAGALES

All species of the genera of the Betulaceae and Fagaceae examined had invariably three bundles and three gaps (*fig.* 4 and *photo.* 1).

It is thus evident that in all those plants grouped originally under the inclusive order Amentiferae the fundamental type of nodal structure is trilacunar. This may occasionally be expanded but in no case is there an approximation and fusion of the three gaps into one.

URTICALES.

All the Ulmaceae and Urticaceae examined agreed in the possession of a trilacunar node. This seems also to be characteristic of most of the Moraceae, although in Ficus there are usually five bundles and gaps.

PROTEALES

In the six genera of Proteaceae examined the nodal structure was invariably trilacunar.

SANTALALES

In the Santalaceae and Loranthaceae there is apparently but a single bundle and gap to each node, but a trilacunar condition is characteristic of the Olacaceae and is therefore probably ancestral for the order as a whole.

ARISTOLOCHIALES

The node of Aristolochia is trilacunar, but the bulk of the vascular supply to the leaf is contained in the central bundle. The two lateral ones are very small and arise close together on the *opposite* side of the stem from that where the leaf is attached, pass around the stem and thus enter the base of the leaf.

POLYGONALES

We have already spoken of the Polygonaceae as a typically multilacunar family. In Mühlenbeckia, Coccoloba, Fagopyrum and most species of Rumex and Polygonum examined there are a considerable number of strands passing off into the sheathing base of the leaf from the entire periphery of the stem (*fig.* 6). In young plants of these forms, however, there are only three bundles to each leaf, and this trilacunar condition, which also prevails in the dioecious species of Rumex (*fig.* 5) and the climbing species of Polygonum, is probably primitive for the order.

CENTROSPERMAE

In this great order a large number of species were investigated in all families save the Cynocrambaceae and Bassellaceae, and in every instance the departure of the foliar strand was found to cause but a single gap in the cylinder. In the Chenopodiaceae, Amarantaceae, Nyctaginaceae and Phytolaccaceae the stem structure is often anomalous, but even here the complex leaf-trace comes off from one definite region and is not inserted in various places around the stem (*figs.* 8 and 10). The simpler species in these groups (*fig.* 9), however, and the very young plants (*fig.* 7) display a single vascular ring with no anomalous bundles. Although the leaf-trace in such forms and in all the rest of the order (the Aizoaceae, Portulacaceae and Caryophyllaceae) never leaves but a single gap, it is almost always three-parted or three-lobed, thus indicating its probable origin from an approximation of the three strands of a trilacunar type. The fact that such a very large series of plants are characterized without exception by a single type of nodal structure is strong evidence for placing them together, especially since the unilacunar condition which they display is possessed by very few of the other lower Archichlamydeae. The Polygonales, with which some or all of the Centrospermae have frequently been included, even in the recent system of Hallier, possess a nodal anatomy so unlike that of the Centrospermae as to preclude any very close relationship, if the structure of the node is to be regarded as of much taxonomic value. Evidence from this region certainly supports the view that the Centrospermae as defined by Engler constitute a very natural order which is somewhat removed from the other lower Archichlamydeae.

RANALES.

The nodal structure of the Ranales is extremely various but seems to have been primitively trilacunar. The three genera Trochodendron, Tetracentron and Euptelea, included by Engler under the family Trochodendraceae, are characterized, respectively, by five, three and one traces and gaps. The Cercidiphyllaceae (Cercidiphyllum) are trilacunar. Among the Ranunculaceae, the presumably more primitive sections of the family (the Paeonieae and Helleboreae) are for the most part trilacunar. Such a condition distinguishes Paeonia (*fig.* 11), Caltha, Coptis, Delphinium and sometimes Aquilegia. Forms in which the primitively follicular ovary has been much reduced, as Actaea, Xanthorrhiza (*fig.* 12 and *photo.* 5) and Cimicifuga, have the base of the leaf supplied with a very large number of strands inserted separately around most of the periphery of the stem. Such a condition is present also in the Anemoneae (Ranunculus, Anemone and Thalictrum), which from their single-seeded fruits are regarded by Engler as the most highly specialized members of the family. Clematis is the only member of this subfamily with a trilacunar node. The trilacunar condition therefore seems to have been primitive for the Ranunculaceae; and reduction of the ovary and amplification of the nodal system have apparently progressed together. The Lardizabalaceae (Akebia and Sinofranchetia) are trilacunar. Among the Berberidaceae, *Berberis communis* and many other species are trilacunar but others, particularly *B. aquifolium*, may have as many as eleven bundles passing off to the leaf base. In Epimedium there are seven strands and gaps. The Menispermaceae seem to be exlusively trilacunar. The Magnoliaceae are perhaps more variable in nodal structure than any other family of dicotyledons. Illicium, Schizandra and Kadsura are unilacunar; Drimys and Tetracentron are trilacunar, and Liriodendron, Magnolia and Michelia are multilacunar. The Calycanthaceae are all trilacunar. The rest of the sub-order Magnoliineae which were observed (the Anonaceae, Myristicaceae, Monimiaceae, Lauraceae and Hernandiaceae) are entirely unilacunar, but in every case the leaf-bundle is three-lobed or tripartite (*photo.* 3), indicating that the nodal type was originally trilacunar.

The nodal structure of the Ranales seems to have been primitively trilacunar and to have progressed both toward amplification and toward reduction. The group as a whole is more variable in this respect than are most orders, a circumstance which seems to favor the idea that it is relatively primitive in constitution, for it is among primitive types that any structure is apt to be found in its most plastic and variable condition.

Rhoedales

In this order the Papaveraceae seem to be invariably trilacunar, but it is noteworthy that the three bundles, at least in the smaller portions of the stem, come off very close together. The Capparidaceae are entirely unilacunar. The Cruciferae, as we have noted above, are both trilacunar and unilacunar (*figs.* 13 and 14), and from the number of intermediate conditions observable, this family furnishes one of the best examples of the way in which one type has been derived from the other. The Resedaceae are unilacunar.

In this order, therefore, the primitive condition was apparently one with three gaps and traces (since it occurs in the most generalized and primitive family, the Papaveraceae) and the whole tendency in the evolution of the group seems to have been toward the approximation and fusion of the various bundles constituting the foliar supply.

Rosales

Plants belonging to this order possess a nodal structure which is almost invariably trilacunar (*figs.* 15 and 17). Some of the more important exceptions observed were the following:—Schizophragma, Astilbe and a few species of Hydrangea, among the Saxifragaceae, have five or seven bundles and gaps; Spiraea, (*fig.* 16), Exochorda and their immediate allies among the Rosaceae are unilacunar and *Potentilla palustris* is multilacunar; among the Crassulaceae, all species of Sedum and Crassula investigated were unilacunar, but in Cotyledon there were in addition to the central bundle two small lateral ones; Eucommia is unilacunar; Platanus has seven bundles and gaps, and Phaseolus, Dolichos, Gymnocladus and a few other genera of the Leguminosae possess a foliar supply of five bundles. Evidence from the node, therefore, indicates that the Rosales are a good natural order.

Geraniales

The Geraniales resemble the Rosales in possessing, for the most part, a trilacunar nodal structure. This characterizes without exception all the members of the Geraniaceae, Tropaeolaceae, Oxalidaceae, Linaceae and Rutaceae which were observed. The Simarubaceae

(Ailanthus) possess seven bundles and gaps, and the Burseraceae and Meliaceae five. Erythroxylon is unilacunar. The Malpighiaceae are prevailingly trilacunar but a few genera (Bannisteria) have but a single gap. The Vochysiaceae, Tremandraceae and Polygalaceae are unilacunar. The large family Euphorbiaceae is prevailingly trilacunar, but certain genera (Ricinus) have more than three bundles and a few (Antidesma) only one. The order agrees with the great majority of Archichlamydeae in being fundamentally trilacunar.

SAPINDALES

Under the Sapindales are included a large number of sub-orders some of which are not very closely related to one another, and the anatomy of the node is consequently rather diverse among the various members of the order.

The sub-orders Empetrineae, Buxineae, and Coriariineae are evidently entirely unilacunar. Among the families included under the Celastrineae both types are represented; the Cyrillaceae and Celastraceae (*fig.* 18) being unilacunar and the Staphyleaceae trilacunar. Most of the Aquifoliaceae (Nemopanthus, Byronia and most species of Ilex) (*fig.* 20) are unilacunar but, as we have previously noted, *Ilex opaca* (*fig.* 19) has three bundles and gaps. The Aceraceae, Hippocastanaceae and Sapindaceae, included under the Sapindineae, are all trilacunar so far as observed save for a few species of Aesculus where there may be five bundles and gaps. The Balsaminaceae are entirely unilacunar but the single trace is clearly tripartite.

It is within such rather heterogeneous groups of plants as are included among the Sapindales that the anatomy of the node, which is so constant within individual families, will probably be found to be of much value in determining the composition and relationship of the various sub-orders. As at present constituted, the Sapindales apparently cannot be regarded as a very "natural" order.

RHAMNALES

In this order the Rhamnales are entirely trilacunar, but the Vitaceae examined have three, five or seven bundles and gaps.

MALVALES

The various families included under the Malvales seem to be very similar in their nodal structure, the Elaeocarpaceae, Tiliaceae, Malvaceae and Sterculiaceae being characterized almost exclusively by a trilacunar node. The only exceptions noted to this were among a few of the Malvaceae which possessed five or more strands.

PARIETALES

The Parietales are another rather large and heterogeneous order. The families included by Engler under the Theineae are various in their nodal anatomy; the Eucryphiaceae and Ochnaceae being trilacunar, the Dipterocarpaceae having three or five bundles and gaps and the Marcgraviaceae, Theaceae and Guttiferae (*fig.* 21) being entirely unilacunar. The Dilleniaceae present another good example of a family which is intermediate between a trilacunar and a unilacunar condition. The genera Dillenia, Tetracera, Davilla, Curatella, Doliocarpus and Hibbertia have either three or five bundles and gaps at the node. They all belong to the sub-family Dillenioideae which, from the simple structure of the stamens and the partial freedom of the carpels, is with little doubt to be regarded as more primitive than the rest of the family. The other two sub-families, the Actinidioideae and Saurauioideae, are more specialized florally and the two genera Actinidia and Saurauia which compose them are both unilacunar in nodal structure. The Dilleniaceae thus present further evidence that the unilacunar condition of the node has been derived from a trilacunar one. All the Cistaceae examined are unilacunar, but the Bixaceae, included with them under the sub-order Cistineae, are trilacunar. All members of the Flacourtiineae investigated (Violaceae, Flacourtiaceae, Stachyuraceae and Passifloraceae) were trilacunar. The Caricaceae have from three to many bundles and gaps at the node. The number in the Begoniaceae is usually five.

OPUNTIALES

The Cactaceae are so much reduced that nodal anatomy is of little value in determining relationships.

MYRTIFLORAE

Aside from the Centrospermae, the Myrtiflorae are the largest order of Archichlamydeae to be overwhelmingly unilacunar. Most of the families grouped under the order by Engler were investigated and in all but three the departure of the foliar supply caused but a single gap in the cylinder (*fig.* 22 and *photo.* 4). These three are the Nyssaceae, Alangiaceae and Rhizophoraceae, which are trilacunar. The first two have often been included under the Cornaceae, an affinity which the structure of the node supports. Nodal anatomy also indicates that the Rhizophoraceae should not be placed in the Myrtiflorae which, aside from these three families, seems to be a natural order.

UMBELLIFLORAE

The Umbelliflorae of Engler include the families Araliaceae, Umbelliferae and Cornaceae. As we have previously noted, the first two of these families are almost invariably characterized by an amplified nodal structure, in which a large number of bundles, each leaving a separate gap, enter the base of the leaf (*fig.* 23 and *Photo.* 6). The Cornaceae, on the other hand, as far as has been observed are always trilacunar (*Photo.* 2). The anatomy of the node thus supports evidence from other sources which goes to show that this family should not be included in the Umbelliflorae but should be placed elsewhere. The order thus reduced to the Araliaceae and the Umbelliferae seems to be a very natural one.

ERICALES

The various families included under this order (Clethraceae, Pirolaceae, Ericaceae, Epacridaceae and Diapensiaceae) are all unilacunar (*fig.* 24) save the Epacridaceae, in which each of the sheathing leaves is supplied by a considerable number of separate strands. If the anatomy of the node is a sound criterion of relationship, the Epacridaceae cannot be placed very close to the Ericaceae. This may well be an instance, however, where the structure of the flower is more dependable than that of the node.

PRIMULALES

Members of all three families of this order are unilacunar.

PLUMBAGINALES

The single family Plumbaginaceae included under this order appears to be entirely trilacunar.

EBENALES

The four families included under the Ebenales seem to be exclusively unilacunar (*fig.* 27).

CONTORTAE

All genera examined of the five families included by Engler in the Contortae are unilacunar save Menyanthes which (with another genus) composes the sub-family Menyanthoideae of the Gentianaceae. In this case three or five bundles enter the base of each leaf from as many gaps. Either Menyanthes should not be included under the Gentianaceae or else we must believe that its nodal structure has been so modified by its aquatic habitat, which has caused its leaves to become sheathing, that evidence from this region should be disregarded.

In the Ericales, Ebenales and Contortae the single leaf-trace shows no indication of being three-lobed, a fact which may be taken to indicate that the unilacunar condition in these orders has been produced by the loss of the two lateral traces rather than by the approximation of the original three.

TUBIFLORAE

This immense order is characterized almost without exception by a nodal structure which is unilacunar (*figs.* 25 and 26). In the many genera from the sixteen families of this order investigated only Cyrtandra, one of the Gesneraceae, displayed other than this single-gapped condition. Three or five strands and gaps is typical for this genus. Such an exception may be regarded as merely one of the cases where the anatomy of the node is not conservative; or it may be taken as an indication that the Gesneraceae are relatively primitive among the Tubiflorae and connect such families as the Bignoniaceae and Scrophulariaceae with the Rubiales.

The leaf-trace in the Tubiflorae is very often three-lobed or tripartite (*fig.* 25) indicating that it has had its origin as a fusion of the three bundles of the ancestral trilacunar type. On evidence from nodal anatomy the Tubiflorae as defined by Engler appear to be a very natural order.

PLANTAGINALES

Plantago is trilacunar.

RUBIALES

All families in this order save the Adoxaceae were investigated. The Caprifoliaceae, Valerianaceae and Dipsacaceae are entirely trilacunar except for a few instances (Sambucus and others) where they may be five bundles and gaps. The Rubiaceae, however, are overwhelmingly unilacunar, the only exception observed being the genus

Sarcocephalus where the presumably ancient trilacunar condition persists.

CUCURBITALES

The Cucurbitaceae are entirely trilacunar, apparently. The petiole in most members of the family is large, especially at the base, and contains a ring of many strands. Just as these enter the stem, however, they become grouped into three bundles, each of which is inserted separately.

CAMPANULATAE

In this order the Campanulaceae are entirely unilacunar. The Goodeniaceae and Compositae, however, are trilacunar (*fig.* 28) or in rather rare cases multilacunar. The theory so generally held and maintained in the recent classifications of Engler and Hallier that the Compositae have been derived from the Campanulaceae or their near allies therefore receives no support from nodal anatomy for if our general hypothesis as to the origin of the different types of nodal structure in the angiosperms is correct, we should certainly not expect the trilacunar condition of the Compositae to have been derived from the unilacunar (and hence reduced) one which characterizes the Campanulaceae. We should more naturally look to the trilacunar Goodeniaceae, Dipsacaceae or Caprifoliaceae for the ancestors of the Compositae. Evidence from nodal anatomy seems to indicate that the Campanulatae ought perhaps to be divided into more than one order.

The following table presents a more condensed summary of the occurrence of the various types of nodal anatomy throughout the dicotyledons. Numbers refer to number of gaps. Those in parenthesis indicate rare conditions for the family.

This tabular review of the nodal anatomy of the dicotyledons makes it evident that we are here dealing with a character which is almost always very constant within any particular family. It will be noted that a trilacunar condition may frequently become expanded into a multilacunar one in the same family or even in the same genus, but that it is rarely contracted into the unilacunar condition in nearly related forms. The two main types are really the unilacunar and the multilacunar and it is only in comparatively few cases, such as the Magnoliaceae, Cruciferae and Dilleniaceae that these both occur in any considerable number in the same family.

VERTICILLATAE
Casuarinaceae, 1.

PIPERALES
Piperaceae, 3 and 7.
Chloranthaceae, 3 and many.

SALICALES
Salicaceae, 3.

GARRYALES
Garryaceae, 3.

MYRICALES
Myricaceae, 3 and (1).

LEITNERIALES
Leitneriaceae, 3.

JUGLANDALES
Juglandaceae, 3 and (5).

JULIANALES
Julianaceae, 3.

FAGALES
Betulaceae, 3.
Fagaceae, 3.

URTICALES
Ulmaceae, 3.
Urticaceae, 3.
Moraceae, 3 and 5.

PROTEALES
Proteaceae, 3.

SANTALALES
Santalaceae, 1.
Olacaceae, 3.
Loranthaceae, 1.

ARISTOLOCHIALES
Aristolochiaceae, 3.

POLYGONALES
Polygonaceae, many.

CENTROSPERMAE
Chenopodiaceae, 1.
Amarantaceae, 1.
Nyctaginaceae, 1.
Phytolaccaceae, 1.
Aizoaceae, 1.
Portulacaceae, 1.
Caryophyllaceae, 1.

RANALES
Trochodendraceae, 1, 3, and 5.
Cercidiphyllaceae, 3.
Ranunculaceae, 3 and many.
Lardizabalaceae, 3.
Berberidaceae, 3 and many.
Menispermaceae, 3.
Magnoliaceae, 1, 3, and many.
Calycanthaceae, 3.
Anonaceae, 1.
Myristicaceae, 1.
Monimiaceae, 1.
Lauraceae, 1.
Hernandiaceae, 1.

RHOEDALES
Papaveraceae, 3.
Capparidaceae, 1.
Cruciferae, 3 and 1.
Resedaceae, 1.

ROSALES
Crassulaceae, 3 and 1.
Saxifragaceae, 3 and (5).
Pittosporaceae, 3.
Brunelliaceae, 3 and 5.
Cunoniaceae, 3.
Hamamelidaceae, 3.
Eucommiaceae, 1.
Platanaceae, 7.
Crossosomataceae, 3.
Rosaceae, 3 and (1) and (5).
Connaraceae, 3.
Leguminosae, 3 and (5).

GERANIALES
Geraniaceae, 3.
Oxalidaceae, 3.
Tropaeolaceae, 3.
Linaceae, 3.
Erythroxylaceae, 1.
Zygophyllaceae, 3.
Rutaceae, 3.
Simarubaceae, 7.
Burseraceae, 5.
Meliaceae, 5.
Mapighiaceae, 3 and (1).
Vochysiaceae, 1.
Tremandraceae, 1.
Polygalaceae, 1.
Euphorbiaceae, 3 and (1).

SAPINDALES
Buxaceae, 1.
Empetraceae, 1.
Coriariaceae, 1.
Anacardiaceae, 3.
Cyrillaceae, 1.
Aquifoliaceae, 1 and (3).
Celastraceae, 1.

Staphyleaceae, 3.
Aceraceae, 3.
Hippocastanaceae, 3 and (5).
Sapindaceae, 3.
Balsaminaceae, 1.

RHAMNALES
Rhamnaceae, 3.
Vitaceae, 3, 5, and 7.

MALVALES
Elaeocarpaceae, 3.
Tiliaceae, 3.
Malvaceae, 3 and (many).
Sterculiaceae, 3.

PARIETALES
Dilleniaceae, 3 and 1.
Eucryphiaceae, 3.
Ochnaceae, 3.
Marcgraviaceae, 1.
Theaceae, 1.
Guttiferae, 1.
Dipterocarpaceae, 3 and 5.
Cistaceae, 1.
Bixaceae, 3.
Violaceae, 3.
Flacourtiaceae, 3.
Stachyuraceae, 3.
Passifloraceae, 3.
Caricaceae, 3 and many.
Begoniaceae, 5.

MYRTIFLORAE
Peneaceae, 1.
Oliniaceae, 1.
Thymeleaceae, 1.
Eleagnaceae, 1.
Lythraceae, 1.
Punicaceae, 1.
Lecythidaceae, 1.
Rhizophoraceae, 3.
Nyssaceae, 3.
Alangiaceae, 3.
Combretaceae, 1.
Myrtaceae, 1.
Melastomataceae, 1.
Oenotheraceae, 1.

UMBELLIFLORAE
Araliaceae, many.
Umbelliferae, many.
Cornaceae, 3.

ERICALES
Clethraceae, 1.
Pirolaceae, 1.
Ericaceae, 1.
Epacridaceae, many.
Diapensiaceae, 1.

PRIMULALES
Theophrastaceae, 1.
Myrsinaceae, 1.
Primulaceae, 1.

PLUMBAGINALES
Plumbaginaceae, 3.

EBENALES
Sapotaceae, 1.
Ebenaceae, 1.
Symplocaceae, 1.
Styracaceae, 1.

CONTORTAE
Oleaceae, 1.
Loganiaceae, 1.
Gentianaceae, 1 and (many).
Apocynaceae, 1.
Asclepiadaceae, 1.

TUBIFLORAE
Convolvulaceae, 1.
Polemoniaceae, 1.
Hydrophyllaceae, 1.
Borraginaceae, 1.
Verbenaceae, 1.
Labiatae, 1.
Solanaceae, 1.
Scrophulariaceae, 1.
Bignoniaceae, 1.
Orobanchaceae, 1.
Gesneraceae, 1, (3) and (5).
Columelliaceae, 1.
Lentibulariaceae, 1.
Globulariaceae, 1.
Acanthaceae, 1.
Myoporaceae, 1.

PLANTAGINALES
Plantaginaceae, 3.

RUBIALES
Rubiaceae, 1 and (3).
Caprifoliaceae, 3 and (5).
Valerianaceae, 3.
Dipsacaceae, 3.

CUCURBITALES
Cucurbitaceae, 3.

CAMPANULATAE
Campanulaceae, 1.
Goodeniaceae, 3 and 5.
Compositae, 3 and (many).

The chief importance of a comparative study of the node will consist in providing us with evidence whereby we may group related families together into orders which shall be more natural than the present ones. No one character is, of course, sufficiently constant to be made the sole basis of such a reclassification. The structure of the node is not always invariable, by any means, and further study will doubtless reveal numerous exceptions to the foregoing brief outline and make necessary many changes in it. The value of such a character as this, however, is that, compared with many others, it is extremely constant and very simple.

The present paper, which is based on a study of only about four hundred genera, is but a brief indication of what are some of the main facts which a comparative study of the node brings forth. A very much more thorough and extensive investigation will be necessary before we shall be able to say to just what extent nodal anatomy may be made useful in classification. That it will assume an important position in the final construction of the phylogeny of the angiosperms appears to be reasonably certain.

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Bussey Institution,
Harvard University.

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DESCRIPTION OF FIGURES OF PLATES XXX–XXXIV

Figures are drawn from transverse sections cut just below the node. Xylem of stem cross-lined, of departing leaf-trace bundles solid black.

FIG. 1. PINACEAE, Picea.
FIG. 2. PINACEAE, Agathis (vigorous twig).
FIG. 3. GNETACEAE, Ephedra.
FIG. 4. FAGACEAE, Quercus.
FIG. 5. POLYGONACEAE, *Rumex Acetosa.*
FIG. 6. POLYGONACEAE, *Rumex obtusifolius.*
FIG. 7. CHENOPODIACEAE, *Chenopodium album* (young plant).
FIG. 8. CHENOPODIACEAE, *Chenopodium album* (mature plant).
FIG. 9. AMARANTACEAE, Gomphrena.
FIG. 10. AMARANTACEAE, Amaranthus.
FIG. 11. RANUNCULACEAE, Paeonia.
FIG. 12. RANUNCULACEAE, Xanthorrhiza.
FIG. 13. CRUCIFERAE, *Sisymbrium leiocarpum.*
FIG. 14. CRUCIFERAE, Barbarea.
FIG. 15. ROSACEAE, Physocarpus.
FIG. 16. ROSACEAE, Spiraea.
FIG. 17. LEGUMINOSAE, Cladrastis.
FIG. 18. CELASTRACEAE, Evonymus.
FIG. 19. AQUIFOLIACEAE, *Ilex opaca.*
FIG. 20. AQUIFOLIACEAE, *Ilex verticillata.*
FIG. 21. GUTTIFERAE, Hypericum.
FIG. 22. MYRTACEAE, Eucalyptus.
FIG. 23. ARALIACEAE, Acanthopanax.
FIG. 24. ERICACEAE, Vaccinium.
FIG. 25. SOLANACEAE, Solanum.
FIG. 26. POLEMONIACEAE, Phlox.
FIG. 27. EBENACEAE, Diospyros.
FIG. 28. COMPOSITAE, Solidago.
FIG. 29. POTAMOGETONACEAE, *Potamogeton pulcher* (after Chrysler).
FIG. 30. ARACEAE, Acorus, young plant (after Chrysler).

DESCRIPTION OF PHOTOGRAPHS OF PLATE XXXV

All sections are transverse, in the region just below the departure of the traces.

PHOTO. 1. *Betula*, trilacunar type. × 20.

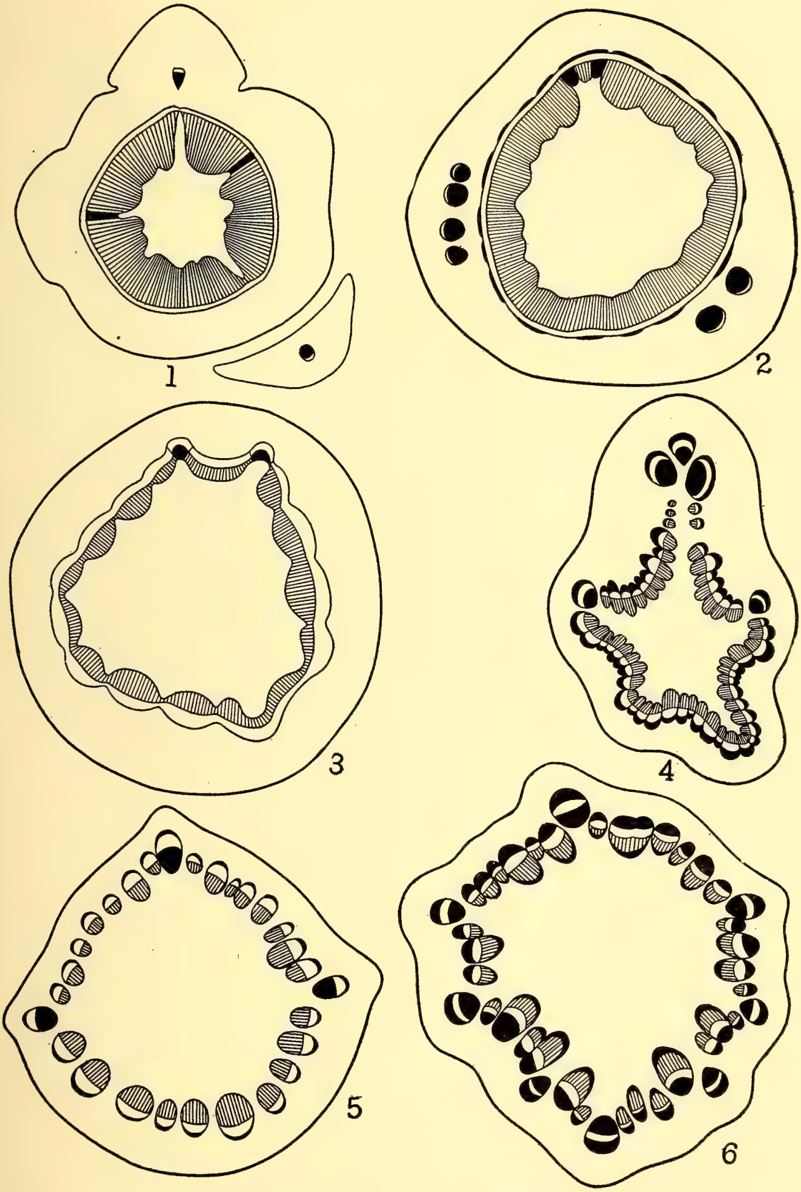
PHOTO. 2. *Cornus*, trilacunar type. × 12.

PHOTO. 3. *Lindera*, unilacunar type. Although the gap is single, there are three bundles in the trace, indicating that the unilacunar condition in this instance has probably arisen by the approximation of three originally distant strands. × 25.

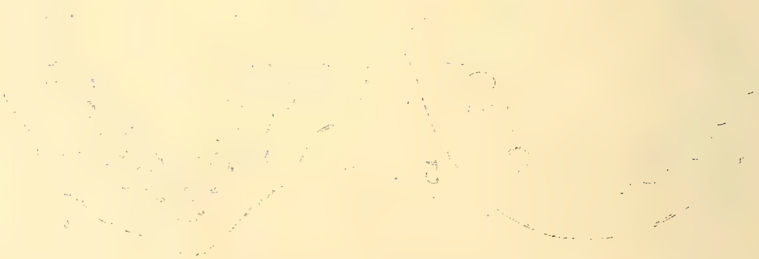
PHOTO. 4. *Eucalyptus*, unilacunar type. In this case the trace is single. × 20.

PHOTO. 5. *Xanthorrhiza*, multilacunar type. × 20.

PHOTO. 6. *Acanthopanax*, multilacunar type. × 9.



SINNOTT: ANATOMY OF THE NODE.



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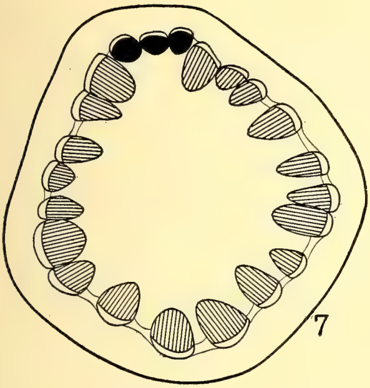

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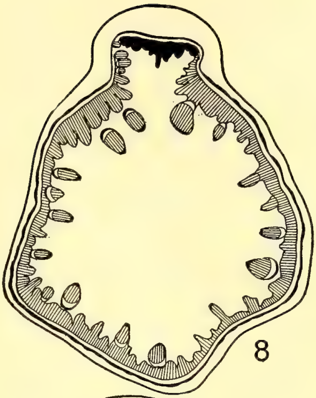

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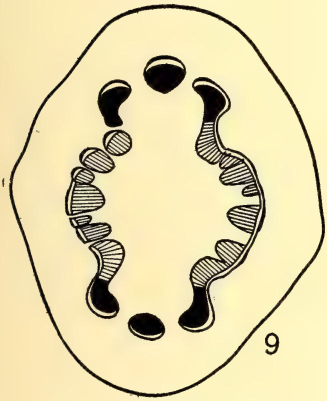

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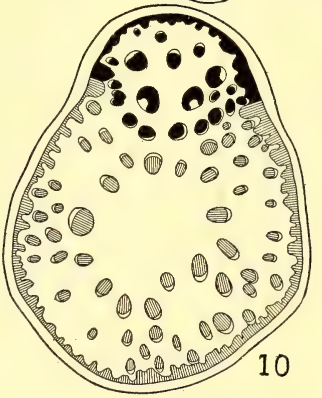

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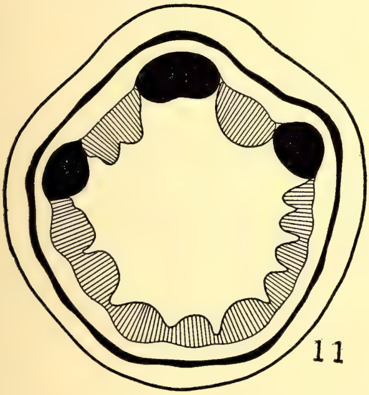

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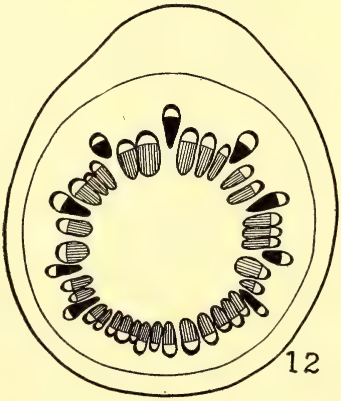

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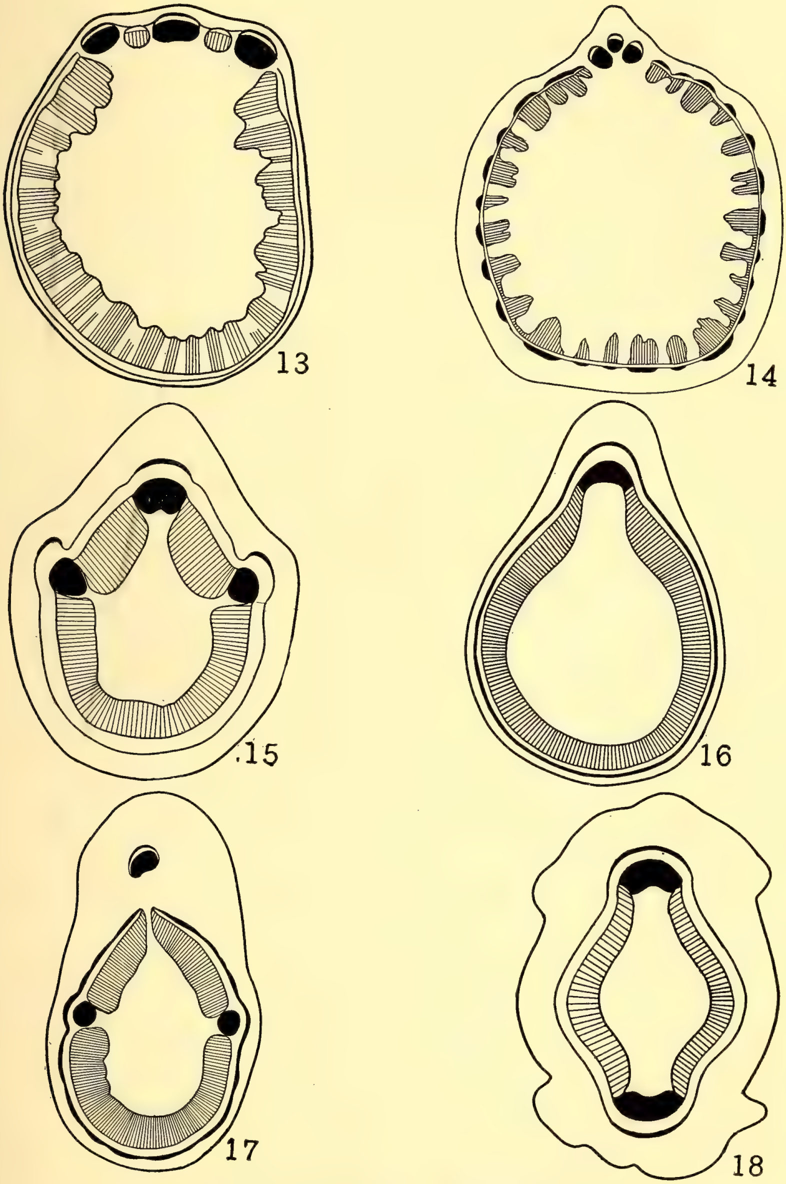
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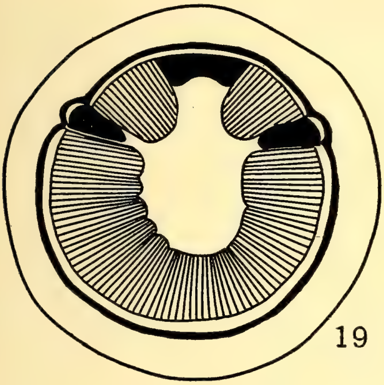

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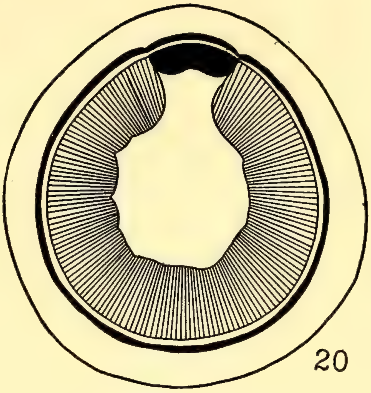

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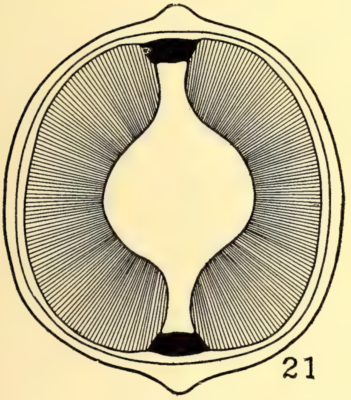

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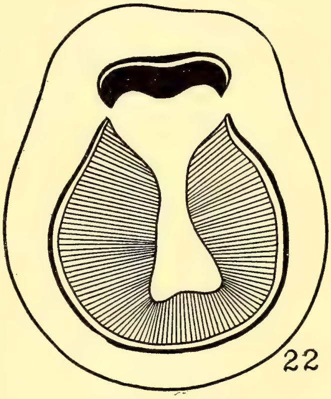

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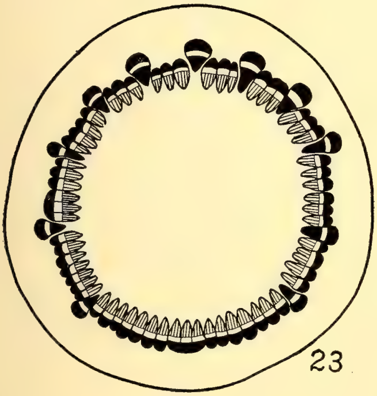

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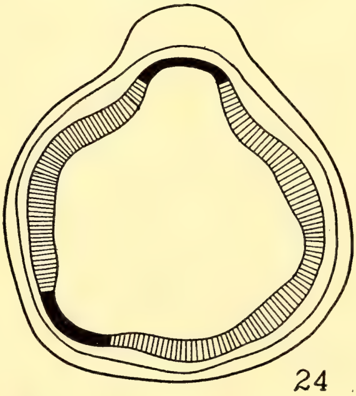

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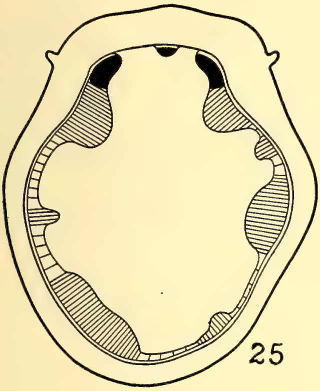
SINNOTT: ANATOMY OF THE NODE.



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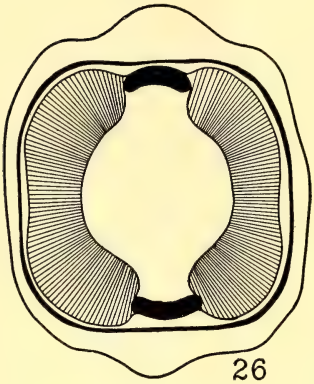

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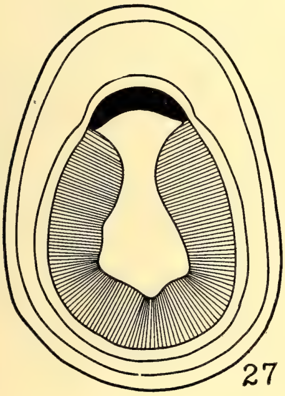

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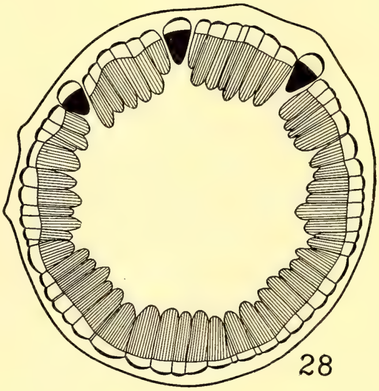

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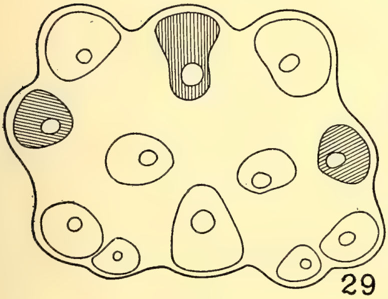

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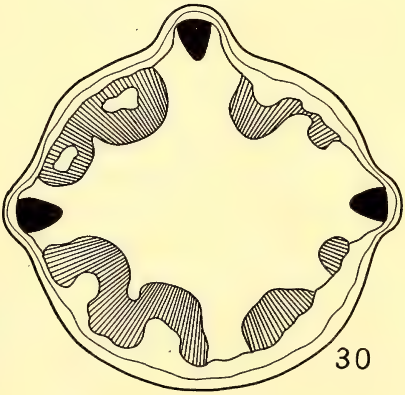

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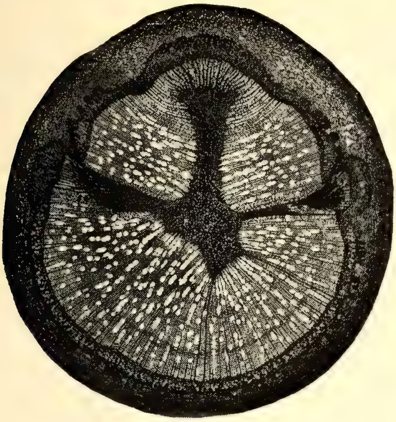
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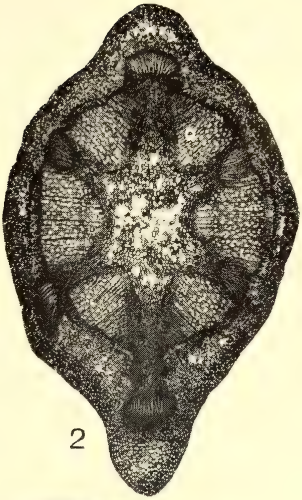
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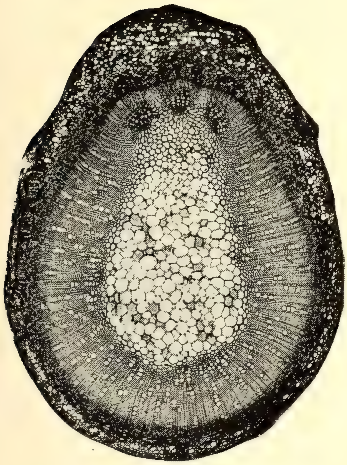
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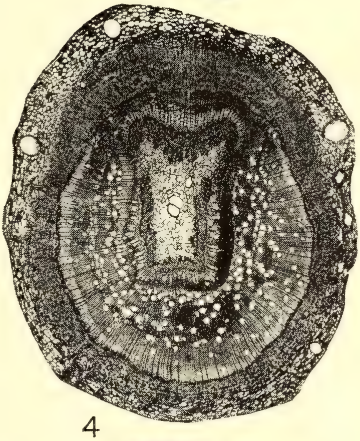
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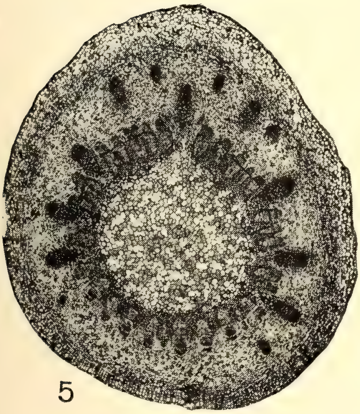
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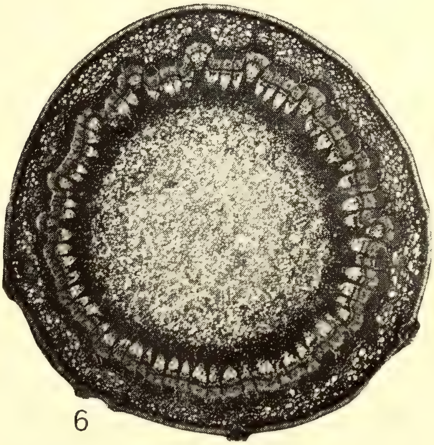
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SINNOTT: ANATOMY OF THE NODE.

STUDIES OF THE DEVELOPMENT OF THE PIPERACEAE

II. THE STRUCTURE AND SEED-DEVELOPMENT OF PEPEROMIA HISPIDULA[1]

DUNCAN S. JOHNSON.

The following study is concerned with *Peperomia hispidula* A. Dietr. This species, which has one of the most peculiar types of embryo sac thus far discovered among angiosperms (Johnson 1907), also has a surprisingly simple vegetative structure, as will be briefly shown here. The whole development of the flower, embryo sac and seed of this *Peperomia* will be described in some detail in order that the development of other species, to be described later, may be compared with it and thus the variety of development found within the genus made evident.

The material used in this investigation was gathered near the Tropical Station of the New York Botanical Garden, at Cinchona, Jamaica, during visits made to that island in 1903, 1906, and 1910. These visits were made possible by grants from the Botanical Society of America, from the Bache Fund, and from the Carnegie Institution of Washington. It is a pleasure to acknowledge here the aid received from these sources and also the courtesies extended by the New York Botanical Garden, in the use of its Cinchona Station, and by the Department of Agriculture of Jamaica. M. Cassimir deCandolle is here thanked for determining the species of this and other *Peperomias* for the author.

The flowers, fruits and vegetative structures of the plant were carefully fixed by the writer, in several mixtures of chromic and acetic acids, in Flemming's fluid, and, in the case of some older seeds, in a mixture of acetic acid and alcohol. The study of the preserved material has been carried on at the Johns Hopkins University and at the Harpswell Laboratory.

We will, for the sake of clearness, arrange the presentation of the features to be discussed under the following heads: *A*. Habit and vegetative structure. *B*. Development of the spike and flower.

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[1] Botanical contribution from the Johns Hopkins University No. 41.

C. The stamen, microspore and pollen tube. *D*. The carpel, fruit, ovule and seed. *E*. The megaspore, embryo sac, embryo and endosperm. *F*. Germination. *G*. Conclusion.

A. Habit and Vegetative Structure

Peperomia hispidula A. Dietr. is a small and very delicate *Peperomia* found (according to deCandolle, Dahlstedt et al.) in Jamaica, Costa Rica, Colombia, Ecuador, Brazil and Argentina. It occurs in damp mountain forests. In Jamaica it is found on the ridge and north side of the Blue Mountains, from 1,600 meters upward. It grows among the mosses and ferns, usually in dense shade, on the nearly saturated humus of the forest floor.

The mature plant is small and decumbent, with the terminal 5 or 6 internodes of each branch assurgent (Swartz 1794, pl. IV). It may flower when only 4 or 5 centimeters long, and the largest plants seen were rarely more than 10 centimeters in length. The stem is delicate, usually whitish or nearly transparent, though, if growing in the light, it is sometimes decidedly greenish. It is sparsely branched, and cylindrical, except for the two ridges extending down each internode downward from the margins of the slightly winged petiole (fig. 10). The internodes are from 30 to 40 millimeters long, and 1 to 1½ millimeters in diameter. At each internode are some 15 to 25 multicellular bristles, similar to those of the leaf blade to be mentioned below. These stiff, tapering bristles consist of 8 to 12 highly vacuolated, thick-walled cells in one longitudinal series, are often a millimeter long and have a diameter of 40 μ (figs. 1, 2). These trichomes stand out in all directions at the node and just above and below it. The stem roots freely by groups of 10 or 12 roots formed near the nodes, and more rarely by scattered roots along the internodes. These roots are only .2 or .3 millimeter in diameter, are but 20 or 30 millimeters long, and are sparsely branched.

The delicate, short petioled, alternately arranged leaves are nearly orbicular, though often somewhat cordate at the base. The larger ones may be 8 or 10 millimeters in diameter and have petioles 3 or 4 millimeters long. The petiole is slightly margined at the sides, and these two wings end against the dorsal side of the lamina just above its base (figs. 1, 14). The two basal lobes of the lamina are connected with each other by a ridge extending across the anterior face of the petiole where it joins the lamina (fig. 1). The insertion of the blade thus approaches the peltate type. Over the upper surface of the lamina, chiefly near the edges, are scattered some 10 to 25 bristle-like hairs of 10 to 20 cells each, and of about one millimeter in length (figs. 1, 3). The whole leaf has almost the aspect of that of a large hydrophytic bryophyte. As the hairs mentioned are not easily wetted it seems possible that these and the hairs at the base of the petiole may serve to prevent the adherence of water to the surface of this denizen of very damp habitats. It is also possible that these hairs may serve as an obstruction to small insects creeping over the plant.

The internal structure of the vegetative organs is also relatively very simple. The delicate, sparsely branched roots have a small, thin root-cap (fig. 4). The youngest root hairs are found about two or three diameters of the root behind the apex of the latter. These hairs may reach a diameter of 10 or 12 μ and a length of a millimeter or more (fig. 6). Fungus hyphae enter the root from without and push among and into the cortical cells. These hyphae are little branched, thick-walled, non-septate and from 4 to 5 μ in thickness. Coiled tangles of these fungus threads with beadlike swellings are found in the layer of the cortex just outside the endodermis (figs. 5, 6, 7). In some places the hyphae become greatly swollen to form sac-like expansions that may become 15 μ in diameter and 54 μ long (fig. 8). Whether these hyphae have the function, as well as the appearance, of an endophytic mycorhiza cannot yet be stated with certainty.

The oldest roots seen still had some root hairs on them; the epidermis was intact and the outer walls of its cells were but slightly thickened or cutinized. The cells of the epidermis are 20 or 25 μ in radial and tangential thickness and 50 or 60 μ long (figs. 5, 6). The cortex of the mature root is of 5 or 6 layers of cells, which are 12 to 40 μ in diameter and 100 to 150 μ long. There are numerous intercellular spaces between the cells of the inner layers of the cortex. Many of these are occupied by the fungous hyphae mentioned above (fig. 6). The radial walls of the endodermis are but slightly thickened but are markedly crinkled, as may be seen in a tangential section (figs. 5, 9). These cells are 12 to 20 μ in diameter and 150 μ long.

The whole central cylinder of the mature root is only 90 or 100 μ in diameter and consists of but 40 or 50 cells in cross section (fig. 6). Of these cells two or three are differentiated as water-conducting elements. One, at the center of the stele, is a vessel 12 or 15 μ in diameter and with ring-like and spiral thickenings on its walls. A smaller, ringed duct 7 or 8 μ thick, lies on each side of this (figs. 5, 6). On each side of the plane in which these three ducts lie is seen a small group of phloem elements, apparently containing only one or two sieve tubes each (fig. 6). The whole thus makes up a very simple diarch bundle. The other elements of the stele are thin-walled cells, about 8 or 10 μ in diameter and 100 to 140 μ long. There is no evidence in the oldest roots seen of the formation of any cambium zone, nor even of the development of any secondary xylem or phloem in the bundles (figs. 5, 6). It is evident that in this creeping plant new water-absorbing and water-conducting tissue is added, not by the development of secondary tissue in roots already present but by the formation of entirely new roots on younger portions of the plant. The time of persistence of the roots was not determined.

The internodes of the delicate, sparingly branched stem are also extremely simple in internal structure. The epidermis, as seen in transverse section is made up of a single layer of cells which are about 15–30 μ wide and thick and 40–100 μ long. Interspersed with these are occasional rather cubical oil cells, and the similarly shaped cells which bear, or have borne, the two-celled, knobbed hydathodes (figs. 10, 12, 13). The latter are identical in structure with those shown in various stages of development in the figures of flower and fruit. The outer walls of the ordinary epidermal cells are but slightly cutinized, but this thin cuticle is peculiar in being thrown into numerous minute corrugations (fig. 13). The middle, cuticular layer of the outer cell wall, often 2 μ thick, is much thicker than the outer cutin layer, while the inner cellulose layer has only twice the thickness of the cutin. The outer walls of the oil cells are very similar in thickness and structure to those of the epidermal cells, while the outer walls of the hydathodes are very thin and without distinguishable layers.

The cortex of the mature internode consists, in addition to the epidermis, of four or five layers of thin-walled cells which vary from 30 to 200 μ in diameter and from 150 to 250 μ in length. Small air-spaces are present, especially between the cells of the outer layers. The endodermis consists of but 18 or 20 cells in transverse section. These have a diameter of from 20 to 60 μ and a length of 50 to 70 μ (figs. 10, 11). The radial walls are but slightly thickened, and the crinkling is coarser than that noted in the endodermis of the root. The whole central cylinder of an internode, within the endodermis, is 150 to 250 μ across, and is often flattened in the plane of the leaf next below (figs. 10, 11). The transverse section of the stele consists of only about 150 cells, which vary in diameter from 5 to 25 μ and in length from 20 to 100 μ. There are usually two distinct groups of primary xylem, one near each edge of the flattened bundle (fig. 11). Each group contains from 4 to as many as 8 or 10 ducts which are ringed, or less often spirally thickened. These ducts vary from 10 to 30 μ in diameter and are often 500 μ in length. Between, and arranged around these ducts are thin-walled parenchymatous cells, which may become 75 μ long. A group of 20 to 30 phloem cells is found outside each group of xylem and on the same radius (fig. 11). Each of the phloem groups consists of 6 to 8 sieve tubes, and of rather more numerous cambiform cells with surrounding parenchyma.

In the stem, as in the root, no indication was found of the formation of a cambium ring nor even of any formation of secondary xylem or phloem. The small amount of water-conducting tissue formed in the root and stem of this *Peperomia* is a fact evidently related to its moist habitat. So small a plant, creeping close to the soil, and growing in a very humid air, must lose very little water by transspiration and thus needs to carry but little water in its conducting system. Moreover, that little water needs to be carried but a short distance, because new groups of roots are formed frequently along the stem as it grows, so that every internode or pair of internodes, except those near the apex, has its own direct connection with the water supply in the soil.

As contrasted with the type of conducting system just described we find that the stem of xerophytic species of *Peperomia*, such as *P. reflexa* or *P. verticillata*, have much more highly developed bundles. In the latter, for example, there are 10 or 12 bundles in two concentric rings. Even in the rather delicate, creeping *P. filiformis*, growing in the same damp forests with *P. hispidula*, there is a ring of four bundles in the stele.

The internal structure of the leaf of *Peperomia hispidula* is also simpler than that of any other species of the genus known to the writer.

The petiole, as seen in cross section (fig. 14), is somewhat flattened dorsiventrally and has a slight wing at each edge. The epidermis consists of rather irregular cells with slightly thickened outer walls, interspersed with scattered oil-cells and hydathodes. The wings of the petiole are made up solely of two layers of the epidermis, or, in some portions, of these and a third layer pushed between them (fig. 14). At the top and toward the base of the petiole are found the simple or branched, bristle-like trichomes (figs. 1, 2). There are three, nearly equal, vascular bundles in the petiole, one median and two marginal. Of the latter, one arises on that side of the xylem strand which is on the side of the stem opposite the leaf. The other marginal bundle, together with the median bundle of the petiole and the bundle from the axillary branch, arises from the other xylem strand of the stele. Each bundle of the petiole consists of 40 or 50 cells in cross section, and includes 5 or 6 thickened ducts and a similar number of phloem elements. These bundles are surrounded, in the cross section, by about 100 parenchymatous cells, 25 to 60 μ in diameter and 40 to 70 μ long, between which are numerous, small air-canals (figs. 14, 15).

The blade of the leaf has a pinnately arranged, sparingly branched vascular system, that supports a delicate but bristly lamina of but 3 or 4 cells in thickness (figs. 1, 2, 17). The epidermis of the upper surface consists of irregularly polygonal cells 40 to 60 μ across, from among which arise the ten- or fifteen-celled, bristle-like trichomes (fig. 3). Each trichome rests upon a ring of 5 or 6 surface cells, that is raised slightly above the upper surface of the leaf and is free from the underlying palisade layer. Stomata are wanting from the upper side of the leaf. Oil-cells are about as abundant as on the lower side. Hydathodes are rather less abundant than below. The epidermis of the lower side consists chiefly of wavy-margined cells, often 100 μ long, sparsely interspersed with stomata, oil-cells and hydathodes (fig. 18). The epidermal cells of the margin and of the lower surface of the veins are much elongated, and have oil-cells only scattered among them, except at the glandular tip of the leaf, where hydathodes may occur also (fig. 18). The cutin layer of the epidermal cells is often striate like that of the stem, especially in cells at the edge of the leaf. The average number of stomata in the areas between the veins is about 40 per square millimeter (fig. 18). The form of the guard cells as seen from the surface is of a type rather common among angiosperms (fig. 19). When viewed in cross section the guard cells are seen to be peculiar in having a thin, sharp-edged cutin lip bordering the stoma, much like that shown by Haberlandt ('87, and '09, p. 424, 427) to be characteristic of aquatics or land plants of very moist habitats, such as *Lemna*, *Trianea*, *Fegatella*, etc. (fig. 20). Moreover, this lip is so broad, and the ventral walls of the guard cells next the slit so widely separated in all sections seen, that it seems likely that these stomata are also like those mentioned in being unable to close at all completely. It seems evident that such a stoma would be no serious disadvantage to a plant of very humid habitats. It is possible that Haberlandt's suggestion, that the sharp-edged guard cells of the plants mentioned prevent closing of the stomata by water drops, may also be applicable to this *Peperomia.* The only portions of the lower side of the leaf where stomata are more abundant than is indicated above are the areas underlying the glandular swellings of the veins near the margin of the leaf (*G*, in fig. 1). Here the stomata become relatively numerous, though apparently not differing in size or structure from the stomata occurring on the thinner portions of the lamina (figs. 17, 18, 21). It is probable that these stomata serve as water stomata, as we shall see later, when speaking of the glands with which they are associated.

The mesophyll portion of the lamina, between the veins, consists of a single layer of slightly specialized, nearly isodimensional palisade cells, and of one or, near the veins, of two layers of sponge parenchyma (figs. 16, 17). Near the margin of the lamina the tissue between the upper and lower epidermis may consist solely in addition to the single layer of palisade, of one layer of very scattered parenchyma cells (figs. 17, 21). The palisade cells are 40 to 45 μ in vertical length and vary from 20 to 45 μ in diameter. Each contains a single large nucleus and from 15 to 25 chromatophores. These cells are closely in contact with other cells except at their rounded inner ends, which are surrounded by considerable air-spaces and rest upon one or more of the underlying parenchyma cells. The latter are often 10 to 15 μ thick and 40 or 50 μ long tangentially, and may contain 12 or 15 chromatophores, which are, however, only about half as bulky as the chromatophores of the palisade cells. It is evident, from their contents and from their relation to other tissues, that these sponge-parenchyma cells must serve not only for photosynthesis but likewise for the conduction of the products of this process from the palisade cells to the vascular bundles. They must evidently also carry water in the reverse direction. The water-storing hypodermis, characteristic of the leaves of all other *Peperomias* studied except those of *P. pellucida* and *P. tenera* (Jäderholm, 1898) is entirely wanting in the leaf of *P. hispidula.* Moreover, there is no evidence from the structure of the epidermis, that this latter tissue serves for water-storage in our species, as Jäderholm says it does in *P. pellucida.* In the leaf of *P. hispidula* then it is evident that we have the mesophyll structure of the aerial, dicotyledonous leaf reduced to about its simplest possible terms.

The vascular system of the lamina of *Peperomia hispidula* is derived from the three vascular bundles of the petiole, which enter the blade separately. The medium bundle forms the midrib, which gives rise from its upper half to two pairs of lateral veins (fig. 1). Each of the two lateral bundles from the petiole forks soon after entering the blade, and the two divisions so formed are connected with each other and with the branches from the midrib in the manner shown in figure 1. The submarginal vein arising from these fusions contains, for most of its length, only three or four vascular elements, and the leaf is but slightly increased in thickness in this region (fig. 17). Each of the three veins entering the base of the blade contains, on the contrary, several times this amount of vascular tissue, and each, with the tissue about it, forms a rib two or three times the thickness of the rest of the lamina. The tissue of these ribs, outside the vascular strands, consists generally of slightly modified parenchyma, surrounded by an epidermis with a few oil cells scattered through it (fig. 16). At the end of the midrib, however, and at one other point on each lateral margin of the blade, there is a broader, more massive thickening of the tissue about the veins. At these points the thickening projects chiefly on the upper side of the lamina (figs. 1, 18, 21). These swellings may be 10 or 11 cells thick, and consist of rounded parenchymatous cells surrounded by intercellular spaces connecting quite directly with those immediately below the numerous stomata (figs. 18, 21). The latter are grouped, without regularity of orientation, over the whole of this glandular swelling (fig. 21). The rounded cells of the swelling have few chloroplasts, large vacuoles and rather large nuclei. The whole structure of the thickenings is closely similar to that described for the water-secreting glands of *Tropaeolum.* Unfortunately the plants brought to Baltimore for the study of these structures in the living *Peperomia* did not live, and I therefore have no experimental evidence concerning the function of these swellings.

Considering the structure of the leaf as a whole, with its non-wetable bristles, its delicate texture, thin cuticle, limited vascular supply, and its well-developed glands evidently fitted for water secretion, it is clear that we have in this *Peperomia* a pretty highly specialized plant. This leaf structure, together with the type of root structure and stem structure noted above, make this species well fitted for the dense, reeking forest which it inhabits.

It is evident from a comparison of this *Peperomia* with the several types in the series of Brazilian species studied by Jäderholm, that *P. hispidula* is a species diverging farthest from the vegetative structure most characteristic of the genus as a whole. The gross and minute structure of the root, stem and leaf of *P. hispidula* are far simpler than those of most species, and are even somewhat simpler than in *P. delicatula* and *P. tenera*, the simplest types thus far described (Jäderholm, 1898). It seems altogether possible that the highly peculiar type of embryo-sac development found in *P. hispidula* is to be attributed in some way to the same environmental conditions in its habitat that seem to have modified its vegetative structure so profoundly.

B. The Development of Spike and Flower

The flowers of *Peperomia hispidula* are arranged in terminal spikes, which may occur in series, one spike opposite each of the four or five terminal leaves of the shoot (figs. 1, 22, 23). The spike consists of from 6 to 14 flowers (commonly there are 9 or 10). When the spike is mature it may, with its stalk, have a length of 15 millimeters (fig. 22). The axis of the mature spike is about half a millimeter in diameter, and the flowers are widely scattered along it. The vascular system of the spike is of a single central bundle, similar to that of the stem (figs. 23, 35). The vascular bundle of each flower runs down through one internode below the insertion of the bract before joining that of the axis (fig. 56).

Each flower consists of a single ovary and two rather long-stalked stamens, all borne in the axil of a short-stalked bract with an orbicular end about half a millimeter in diameter (figs. 22, 25). The lowest bract of the spike generally bears the usual carpel and two stamens above it, but at the tip of the spike are found from one to four or five bract-like outgrowths of the axis, which are entirely without rudiments of either pistil or stamens (fig. 22). Only in rare cases were unisexual flowers found, and these were always pistillate only. The distribution of sexes is therefore much more definite and constant here than in *Piper Betel* (Johnson, 1910, p. 716). The pistils and stamens initiated, however, often fail to produce mature embryo sacs and microspores and usually only 4 or 5 ripe seeds are found on a single spike.

The development of the bract is initiated by the formation of a rounded lateral projection from just below the growing point of the axis (fig. 24). This protuberance soon begins to flatten at the end, and thus ultimately gives rise to the characteristic, short-stalked, orbicular bract. It has an epidermis sprinkled with oil cells and hydathodes, and within is a short, simple branch from the vascular bundle of the axis (figs. 25, 56). This bract reaches essentially its mature size, 125 μ in diameter, before the embryo sac of the same flower is ripe (figs. 22, 56, 78). As the fruit matures the bract shrivels somewhat but remains attached to the axis, even after the fruit has fallen. It is evident that this bract does not, except in the very earliest stages of the flower, protect the latter from desiccation or other injury (figs. 22, 56, 78). This subtending bract of the flower plays a far more important part in shielding the young flower in the cases of the more xerophytic species of *Peperomia*, as we shall have occasion to point out in a later paper.

C. The Development of the Stamen, Microspore and Pollen Tube.

The stamen of *Peperomia hispidula* arises by the outpushing of a group of 8 or 10 periblem cells on each side of the young floral bract, and below the carpel which is developing at the same time just above the bract (figs. 25, 26, 27). When the group of hypodermal cells at the end of the young stamen has reached the number of about 40 or 50, there appear, in two regions where the microsporangia are to be formed, numerous periclinal walls in these cells (figs. 29, 30). The inner of the two layers thus formed gives rise to the definitive archesporium or sporogenous layer of the microsporangium. The outer layer is a primary parietal layer, the cells of which soon divide by periclines into two series. The outer series of cells thus formed constitutes the fibrous layer or endothecium of the mature anther wall. The inner of the two series divides once more by periclines forming the single tapetal layer within and an outer layer, lying next the endothecium, which becomes the innermost layer of the wall of the mature anther (figs. 33, 34). In the mature microsporangium the epidermal cells have thin, uncutinized and collapsed outer walls. The endothecium of the mature anther is about 20 μ thick, and consists of polygonal cells, 20 to 25 μ in diameter, with thin outer walls and thick inner ones. The lateral walls have 15 or 20 rib-like thickenings, which are really radial projections of the basal or inner wall (fig. 34). The nuclei of these cells, like those of the epidermis, degenerate and flatten out as the anthers ripen. The cells of the layer next within the endothecium are represented in the mature stamen only by nearly collapsed cells with degenerating nuclei. The cells of the tapetal layer itself are well-developed at the time the microspore mother-cells are mature, and have dense cytoplasts with large nuclei (fig. 32). With the maturing of the anther the cytoplasts become thin, and the nuclei follow those of the other layers in gradually degenerating.

The opening of the ripe anther is evidently accomplished chiefly by the change in form of the endothecial cells, though this process has not been studied minutely. The rupture of the anther walls occurs along the series of smaller cells at the juncture of the two microsporangia (fig. 34). In sections of the opened anther (fig. 22, above middle of spike) it is seen that the outer walls have bent in and in consequence the anther walls have become completely everted, and the inner surface strongly convex. Soon after the anther is emptied the filament breaks off, leaving only a short stump projecting from the base of the ovary (fig. 22).

From the definitive archesporium, just within the parietal layer, are developed, chiefly by periclinal divisions, the one hundred or more microspore mother-cells of each microsporangium (figs. 31, 32). The sporogenous cells increase in size from a diameter of 8 or 10 μ, when first cut off, to one of 12 to 18 μ at the time of synapsis in the spore mother-cell (figs. 29, 32). The nuclei in the meantime increase from 5 or 6 μ to 10 or 12 μ in diameter, and the bulk of the chromatin also increases slightly up to the time of synapsis. The nucleoli of the definitive archesporial cells are usually single, and do not vary greatly from 2 or 3 μ in diameter in the resting nucleus throughout the development.

It will be well to emphasize at this point the fact that though the number of sporogenous cells in each sporangium varies, as was suggested in the last paragraph, no cases were seen in which the number of microsporangia was greater or less than two. There is thus no evidence from the development that this stamen ever had more than two microsporangia, and no examples were seen of the marked variability in the number of degrees of development of these that occurs in *Piper betel* (see Johnson, 1910). The only suggestions of this are found in those rare cases where the terminal flower of a spike may be pistillate only, and in the fact that from 10 per cent to 25 per cent of the nearly matured microspores in a stamen may prove sterile (fig. 34). The latter condition is not unusual among angiosperms as a whole and therefore is of no special significance in this case. It is surprising that in all of the *Peperomias* thus far studied there is no indication of the derivation of the bisporangiate stamen of this genus from the four-sporangiate stamen possessed by other genera of the Piperaceae, in common with the great bulk of other angiosperms. This loss of all traces of a four-sporangiate phase of the stamen, through which it seems evident that it must have passed in its phylogeny, is an important fact. It will be well for us to have this fact in mind, when later we note the lack of any clear traces in the ontogeny of the phylogenetic origin of the peculiar type of embryo sac found in this genus.

The nuclear phenomena occurring during the division of the sporogenous cells, and during the maturing of the spore-mother cell and its division into the spores, are essentially typical. The appearance of the chromatin net and nucleolus of the resting nuclei, formed during this development, remain practically constant up to the time of preparation for synapsis in the nucleus of the spore mother-cell. During the earlier divisions of the archesporial cells and their nuclei, divisions may not occur at the same time in the different cells (fig. 29). As the time for synapsis approaches, however, the nuclear changes go on at very nearly the same rate in all the mother cells of the same sporangium (fig. 32). The nuclear phases may differ slightly in rate of progress in the two sporangia of the same stamen. The chromatin net of the mother-cell, just before synapsis, is rather large-meshed in parts, and is roughened with many granules (fig. 36). The first evidences of the approach of synapsis are a loosening up of the net and an increase in size, apparently accompanied by a decrease in number, of the chromatin grains, until these become nearly as large and of very closely the same number as the chromosomes of the sporophyte (fig. 37). At this early phase of synapsis the chromatin framework is still distributed about the whole periphery of the nuclear cavity, most of it lying close to the wall. The nuclei of the stamen in the same spike, next older than the stamen whose nuclei show the arrangement just described, have each a tangled chromatin thread that is distinctly contracted away from the nuclear wall. In such a nucleus but few chromatin granules are visible. The completely contracted synaptic knot stage follows very soon after the stage just mentioned, and in it the nucleolus and all of the chromatin, except a few outlying granules are compacted into a rounded mass occupying less than a tenth of the nuclear cavity (figs. 38, 39). As the nucleus emerges from synapsis some of the constituent chromatin threads are loosened up, thus again becoming visible. Some portions of the thread are distinctly beaded (fig. 40). This loosening up of the chromatin thread continues till, from the synaptic knot, a slender, beaded, loosely coiled spireme is formed, which lies chiefly near the periphery of the nuclear cavity. Some loops of this spireme are evidently double (fig. 41). From this time onward the spireme thickens, and where the beading is at all distinct, the chromatin masses are evidently larger. Figure 41, for example, is of a nucleus the thick spireme of which is evidently just about to segment into chromosomes. In the other sporangium of the anther from which the latter figure was drawn the mother-cell nuclei had already divided.

There is nothing peculiar about the spindle formed in the two divisions of the nucleus of the microspore mother-cell. The chromosomes of the first division are about 1 μ thick and 2 or 3 μ long, and are bent to U-shaped or V-shaped bodies so that it is difficult to count them with certainty (figs. 42, 43). The character of the chromosomes before and during this division was not made out in sufficient detail to demonstrate the occurrence of a true reducing division here. The two chromosome groups resulting from this first division become organized into definite nuclei, with a distinct nuclear wall and a coarse peripheral net of chromatin. In the latter there can often be distinguished about 14 larger chromatin grains or masses which range from a half μ to one μ in thickness (fig. 44). Remains of the first spindle are clearly seen even at this stage. The chromosomes formed at the second division are 12 or 14 in number, and are at first much more rounded than those of the first division. Even when they move toward the poles these chromosomes are but slightly elongated and not much bent. The spindle of this second division persists, and by it, together with the remains of the spindle of the first division, the four spore nuclei, when first formed, are connected together (fig. 45).

The microspores of the tetrad are cut out in the usual manner, and the nucleus of each shows then a well-developed, fine-meshed chromatin net (figs. 46, 47). By the time the tetrahedral spore has rounded out to a globular form, and before the exine has become much thickened, the nucleus of the pollen grain divides to two (figs. 48, 49). One of these two nuclei soon becomes twice the bulk of the other (fig. 50). The larger, probably the pollen tube nucleus, lies nearer the wall of the pollen grain. The cytoplasm about the smaller, more centrally placed, generative nucleus becomes denser and less vacuolated than the peripheral cytoplasm. Then a spherical mass of the latter, about half the diameter of the pollen grain, is cut off from the rest of the cytoplasm by a spherical cleavage surface or wall (fig. 50). The exact method of formation of this cleavage wall was not made out. Neither could a definite cellulose wall be demonstrated. In some spores, however, a considerable shrinkage space could be seen between the dense central and the vacuolated peripheral cytoplasm. This central, spherical mass may come into contact with the inner wall of the microspore, but never seems to become appreciably flattened against it, as often happens in the microspores of other angiosperms. This line of separation between the generative cell and the rest of the microspore is still visible in the ripe pollen grain (fig. 51). The nucleus of the generative cell has by this time become equal in size to the pollen tube nucleus. The brownish exine of the ripe pollen grain is greatly roughened by rounded wart-like protrusions, while the intine remains colorless, smooth and about three times the thickness of the exine (figs. 50, 51).

The germination of the pollen grain on the stigma and the fate of the two nuclei mentioned have not been observed. Structures have been seen in the interior of the style that stain differently than the surrounding tissue, and look like pollen tubes, but in no case could anything be made out of the contents of these tubular structures, and their identity as pollen tubes is not absolutely established.

The above account of the development of the stamens and ripe microspores has been given in some detail for the sake of making it evident that there are no clearly primitive features in this phase of the development of this, in many respects, simplest of the *Peperomias*. Some details of the history of the chromatin of the spore nuclei have been given because the sequence of stages can be followed with such certainty in the successive flowers of the spike. It will also be important for us to have these details in mind when discussing the origin of the embryo-sac nucleus, where the occurrence of a typical reduction division is of critical value in interpreting the nature of the mature embryosac.

EXPLANATION OF PLATES XXXVI–XXXVIII

All figures are camera drawings and are from hand sections or microtome sections, except figures 1, 2, 18 and 22.

The magnification given in the description of each figure is that actually shown by the figure as printed on the plate.

Abbreviations used: *A*, air chamber; *As*, archesporium; *Ax*, axis; *Br*, floral bract; *Bs*, bristle of fruit; *C*, carpel; *Cp*, chloroplast; *D*, nuclear disc; *DAs*, definitive archesporium; *E*, egg; *Ed*, endosperm; *EdN*, endosperm nucleus; *Em*, embryo; *En*, endodermis; *F*, fungus hyphae; *FL*, fore-leaf; *H*, hydathode; *In*, integument; *L*, leaf; *Mp*, micropyle; *Nc*, nucellus; *No*, nucleolus; *Oc*, oil-containing cell; *Osp*, oospore; *P*, perisperm; *Pa*, parietal layer; *PG*, pollen-grain; *Pl*, phloem; *PMC*, pollen mother-cell; *PPa*, primary parietal layer; *Pr*, peripheral cell; *S*, stem, *SF*, spindle fibers; *Sg*, stigma; *Sp*, spike; *St*, stamen; *Sy*, synergid; *Tp*, tapetum; *Tr*, trichome; *VB*, vascular bundle; *W*, wing of stem or decurrent margin of petiole.

FIG. 1. Surface view (glycerine mount) of terminal portion of mature plant, showing stem, two spikes and the position and structure of leaves. × 2½.

FIG. 2. Optical section (glycerine mount) of trichome on stem opposite base of leaf. × 117.

FIG. 3. Part of vertical section of leaf, with multicellular trichome. × 117.

FIG. 4. Longitudinal section of tip of primary root. × 117.

FIG. 5. Part of longitudinal section of mature root, showing vascular bundle, crinkled endodermis and cortex. × 216.

FIG. 6. Transverse section of mature root, showing vascular bundle, endodermis, root hairs, and fungus hyphae in cortex. × 117.

FIG. 7. Part of radial section of root, showing penetration of cell walls of cortex by fungus hyphae. × 216.

FIG. 8. Portion of similar section, showing a much swollen hypha. × 216.

FIG. 9. Part of tangential section of root showing crinkled radial walls of endodermis. × 216.

FIG. 10. Transverse section of stem. × 28.

FIG. 11. Transverse section of stele of stem. × 117.

FIG. 12. Part of longitudinal section of young stem (peduncle), showing epidermis with oil cells and hydathodes. × 400.

FIG. 13. Part of longitudinal section of mature stem, showing structure of cell wall of epidermis. × 340.

FIG. 14. Transverse section of petiole. × 36.

FIG. 15. Transverse section of median vascular bundle of petiole. × 252.

FIG. 16. Part of transverse section of leaf including its midrib (glycerine mount). × 117.

FIG. 17. Part of transverse section of leaf at margin (glycerine mount). × 117.

FIG. 18. Part of lower surface of leaf, showing distribution of stomata, hydathodes and oil-cells, overlying water glands at apex of leaf (glycerine mount). × 117.

FIG. 19. Surface view of typical stoma and its surrounding cells. × 216.

FIG. 20. Transverse section of guard cells with cutinized margins (glycerine mount). × 400.

FIG. 21. Longitudinal section of tip of leaf through gland at end of midrib. × 117.

FIG. 22. Surface view of spike, showing young fruits and stamens with unopened and with empty anthers (glycerine mount). × 12.

FIG. 23. Longitudinal section of fruit with terminal spike and two lateral axillary buds. × 27.

FIG. 24. Longitudinal section of apex of a spike, showing growing point of stem and three floral bracts, in the axils of which carpels are being initiated, as is indicated by the bulging and deeper staining of cells. × 166.

FIG. 25. Longitudinal section of floral bract with young ovary. × 216.

FIG. 26. Transverse section of base of bract through young ovary and stamens. × 216.

FIG. 27. Transverse section of stamen of the age shown in last figure. × 405.

FIG. 28. Transverse section of bract with half grown ovary and stamens. × 63.

FIG. 29. Longitudinal section of young stamen. × 540.

FIG. 30. Transverse section of slightly older stamen. × 540.

FIG. 31. Transverse section of stamen showing three-layered wall, definitive tapetal layer, and young pollen mother-cells. × 315.

FIG. 32. Transverse section of stamen with nuclei of pollen mother-cells in synapsis. × 315.

FIG. 33. Longitudinal section of nearly mature stamen. × 148.

FIG. 34. Transverse section of nearly mature stamen. × 148.

FIG. 35. Transverse section of stele of axis of spike, midway its length. × 360.

FIG. 36. Section of mature pollen mother-cell, just before the beginning of synapsis. × 1,300.

FIG. 37. Section of pollen mother-cell at the beginning of synapsis. × 1,315.

FIG. 38. Similar section showing later phase of synapsis. × 900.

FIG. 39. Similar section showing complete synapsis. × 900.

FIG. 40. Similar section showing beginning of recovery of nucleus of pollen mother cell from synapsis. × 1,315.

FIG. 41. Similar section showing beginning of formation of chromosomes. Spireme double. × 1,315.

FIG. 42. Similar section showing part of chromosomes in first division of pollen mother-cell. × 1,315.

FIG. 43. Transverse section of spindle of first division of spore mother-cell, showing form of chromosomes in equatorial plate. × 1,315.

FIG. 44. Section of pollen mother-cell showing the daughter nuclei organized after first division. × 1,125.

FIG. 45. Similar section showing four pollen nuclei still connected by fibers. × 1,315.

FIG. 46. Similar section showing tetrad of young pollen grains. × 900.

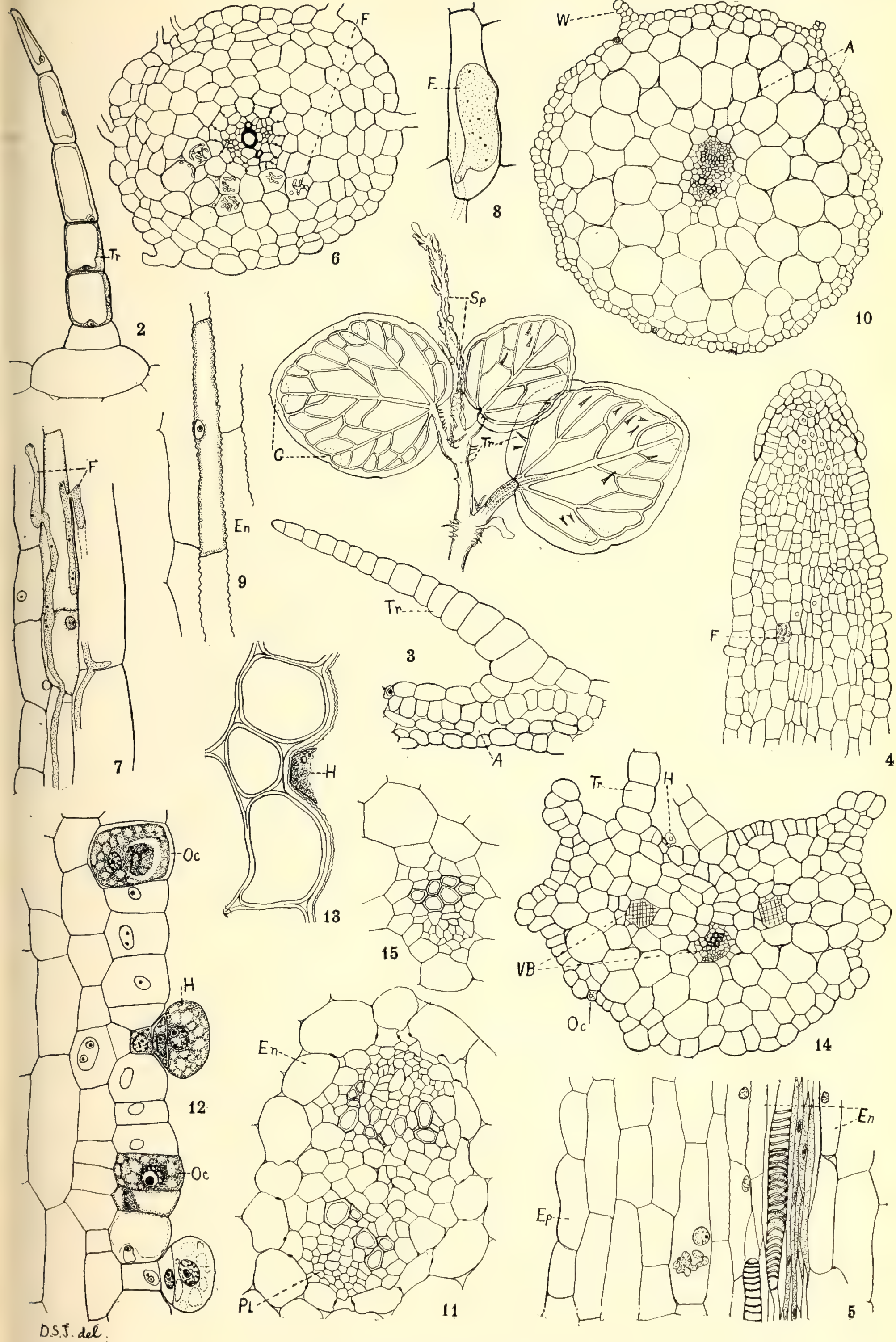
FIG. 47. Section of uninucleate pollen grain, with large central vacuole and partly thickened wall. × 1,315.

FIG. 48. Section of older pollen grain showing numerous small vacuoles. × 1,315.

FIG. 49. Section of pollen grain with spindle for division into pollen tube nucleus and generative nucleus. × 1,315.

FIG. 50. Section of pollen grain showing generative nucleus with some cytoplasm cut off by cell wall. × 1,315.

FIG. 51. Section of mature pollen grain still showing faintly the outline of the generative cell. × 1,315.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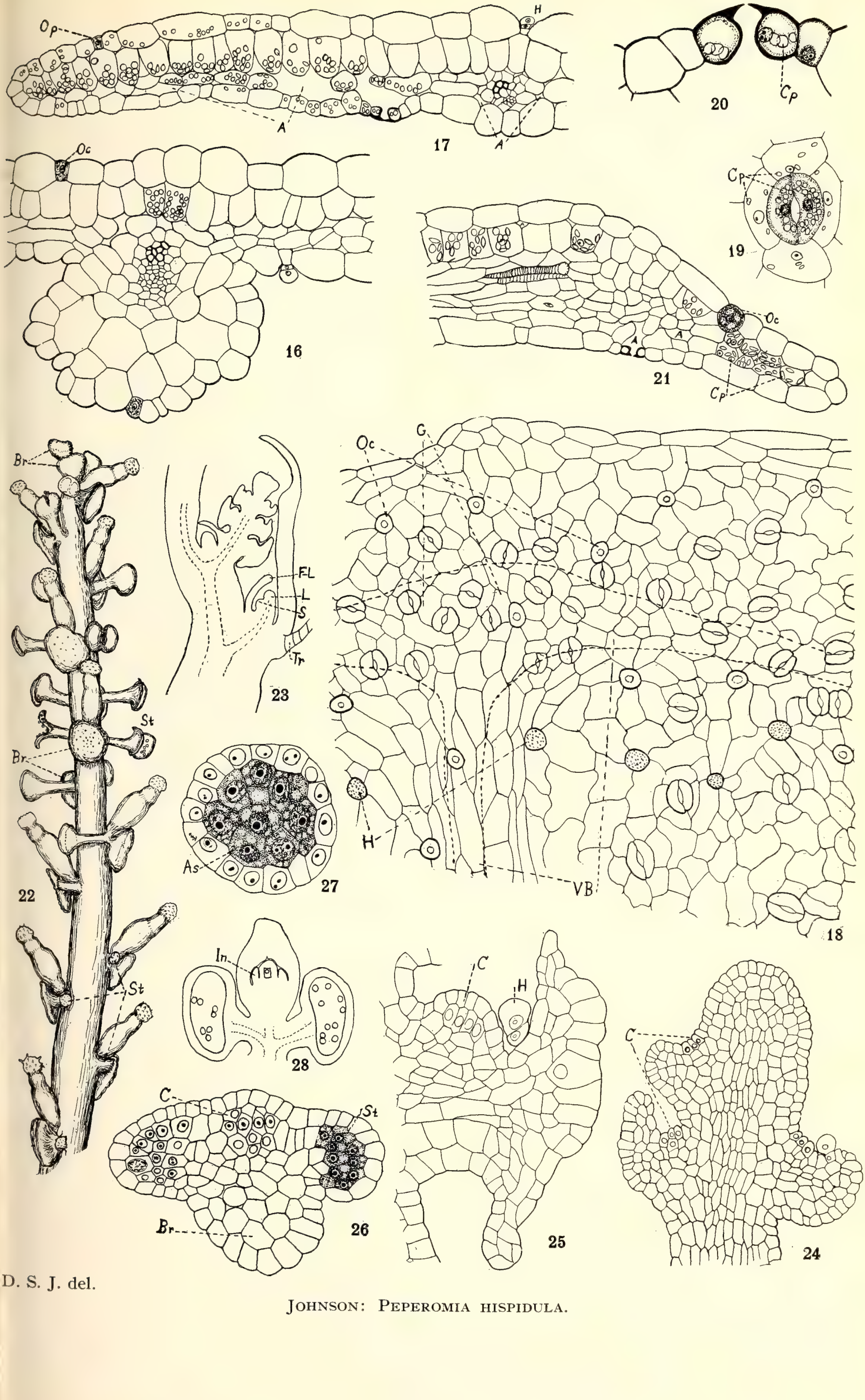
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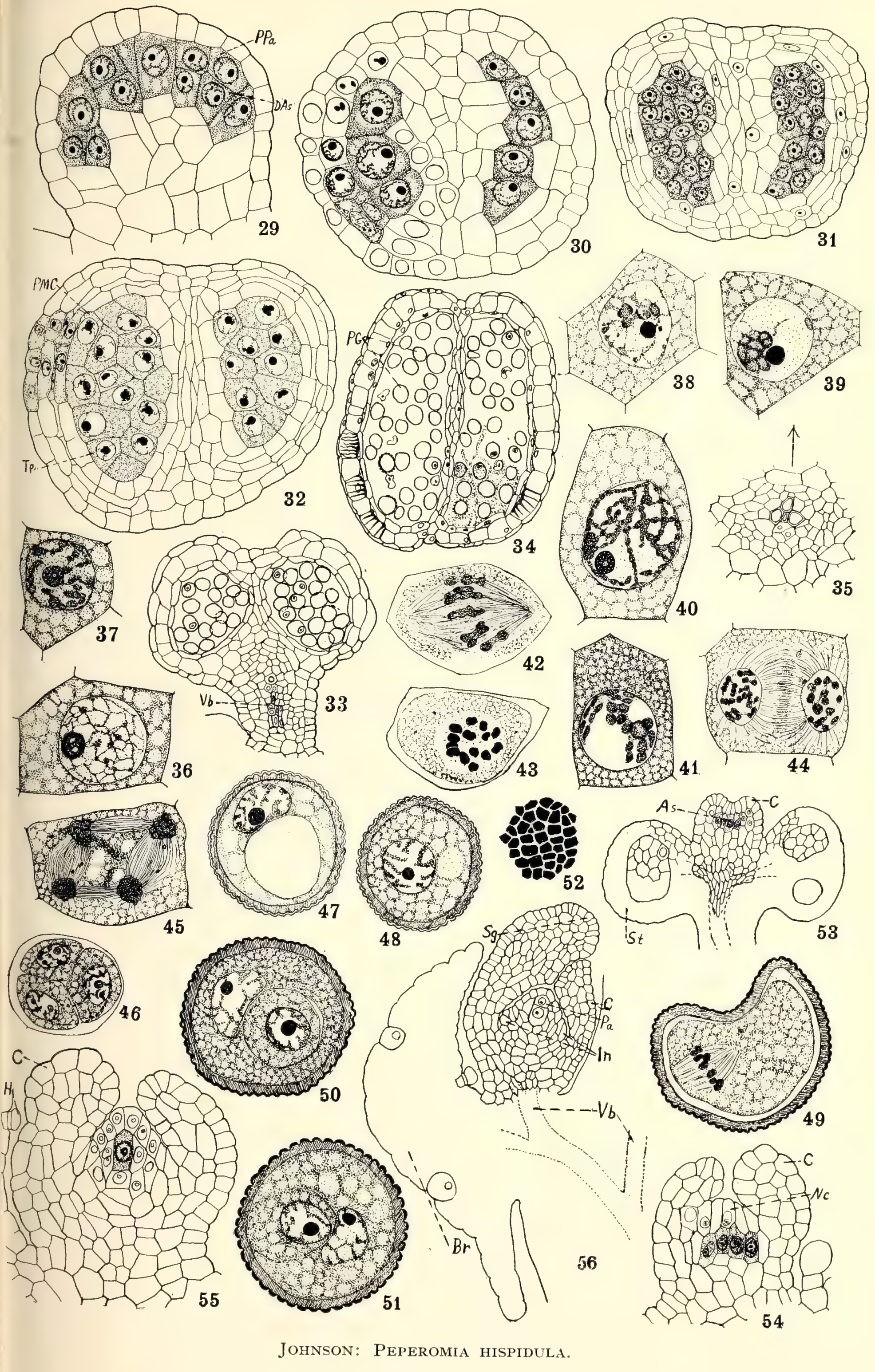
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JOHNSON: PEPEROMIA HISPIDULA.

FIG. 52. Section showing surface of wall of mature pollen grain. × 1,315.

FIG. 53. Approximately longitudinal section through young flower, showing ovule and two stamens. × 148.

FIG. 54. Longitudinal section of young ovary. × 148.

FIG. 55. Longitudinal section of ovary, showing carpels and primary archesporial cell of ovule. × 338.

FIG. 56. Longitudinal section of ovary and subtending bract, showing vascular connection of these with floral axis. Definitive archesporial cell and parietal cell in ovule. × 148.

A GLOEOSPORIUM DISEASE OF THE SPICE BUSH

J. J. Taubenhaus

In June, 1909, Dr. M. T. Cook, formerly of the Delaware Experiment Station, called my attention to a disease of the spice bush fruit. Diseased material was at once collected and cultures made from the interior of the affected fruit, care being taken to observe all the rules of asepsis. In three days a pure culture of a Gloeosporium had appeared in all the poured plates. As far as I could determine from literature, no Gloeosporium disease was reported to attack the spice bush. Shear[1] in his extensive studies on the genus Glomerella does not mention the spice bush as being the host to a Gloeosporium. Ellis and Everhart[2] first described *Gloeosporium officinale* E. & E. on sassafras in Delaware. *Sassafras variifolium* (Salisb.) Ktze. is a shrub, which in common with the spice bush, *Benzoin aestivale* (L.) Nees. belongs to the family Lauraceae. In studying the disease, the possible identity of the spice bush Gloeosporium with that of the sassafras at once suggested itself. Studies were therefore made to determine the pathogenicity of the fungus, its identity with *Gloeosporium officinale* E. & E. and the relationship of both fungi to *Gloeosporium fructigenum* Berk. of the bitter rot of the apple.

Symptoms.—The spots on the green immature spice bush fruit are characterized at first by small darkish depressions. Several of these spots either coalesce and form a larger one, or a single spot gradually enlarges and invades the whole area of the fruit which as a result drops off prematurely. The ascervuli usually appear later when the fruit drops, or within twenty-four hours if placed in a moist chamber. The disease is not confined to the fruit alone, but may attack also the tender foliage and twigs. The symptoms on the plant later are those resembling somewhat the injury due to fire blight of young apple shoots, with the difference, however, that in the spice bush the disease seems to be limited to the tender portions of the plant. Diseased leaves do not seem to form ascervuli while still attached to the plant, but readily develop within twenty-four hours when placed in a moist chamber. A pure culture of the Gloeosporium may be readily obtained from both infected leaves and stems of the spice bush by first sterilizing the surface, then placing bits of tissue in agar plates.

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[1] Shear, C. L. and Wood, A. K. Studies of fungous parasites belonging to the genus Glomerella. Bur. Pl. Ind. Bul. 252, 1913.

[2] Ellis, J. B. and Everhart, B. M. New species of fungi from various localities. Proc. Acad. Nat. Science, Philadelphia: 322–386, 1894.

Pathogenicity.—The method of inoculation was to spray the leaves and fruits of the spice bush with an atomizer containing a heavy suspension of the Gloeosporium spores from a pure culture in sterile water. The inoculated parts were then enclosed in lamp chimneys and both open ends closed with sterile non-absorbent cotton. Checks were treated similarly but they were sprayed with distilled water. Ten chimneys were used at one time, two for the checks and eight for the inoculations. The experiment was repeated three times, and the lamp chimeys were not taken off before twenty-four hours. After each experiment the lamp chimneys were immersed in 5 per cent. formaldehyde for ten minutes, then rinsed in sterile water. Typical infections began to appear on the tender leaves in from three to eight days, but the older leaves remained healthy. The inoculated fruits also showed the typical spotting. All the checks remained healthy. The symptoms obtained from the artificial inoculations were the same and identical with those of the natural infections. The above experiments were carried on in June and July of 1909. In the fall of that same year young spice bush seedlings were dug out in the woods and planted in sterile pots and soil in the laboratory. The seedlings were kept for four weeks. Two dried up and were discarded, eight made a good start and showed no disease. Two of these plants were left as checks and six sprayed with a heavy suspension of the Gloeosporium spores from a pure culture originally isolated from the diseased spice bush fruit. All the eight plants were covered with bell jars for twenty-four hours. A week after infection the tip leaves of the inoculated seedlings turned brown and died, whereas the leaves on the check plants remained healthy. The fungus was readily re-isolated from the artificially infected leaves, and these when placed in a moist chamber for twenty-four hours were covered with a layer of acervuli of salmon-colored spore masses.

Identity of the spice bush Gloeosporium.—I have already mentioned that *Gloeosporium officinale* E. & E. has been reported on sassafras leaves. In pure cultures this fungus and the spice bush Gloeosporium cannot be distinguished from *Gloeosporium fructigenum* Berk. of the apple. Typical infections on the spice bush were obtained with spores of *Gloeosporium officinale.* Similar results were also obtained when the sassafras was inoculated with spores from the spice bush Gloeosporium. The checks remained healthy. This seems to prove that the Gloeosporium from the spice bush and *Gloeosporium officinale* E. &. E. from the sassafras are one and the same. In previous papers I[3] have attempted to show that *Gloeosporium officinale* E. & E. is the same as *Gloeosporium fructigenum* Berk. of the apple, since it produces typical symptoms of bitter rot of the apple and also infects the sweet pea, producing the typical symptoms of anthracnose. Inoculation experiments were carried on with the spice bush Gloeosporium on apples in the orchard, and on sweet pea seedlings. The results obtained were the same as those above stated with *Gloeosporium officinale* E. &. E. This seems to prove that the Gloeosporium from the spice bush is identical with *Gloeosporium officinale* of the sassafras and that both are one and the same with *Gloeosporium fructigenum* Berk. of the apple whose perfect form is known as *Glomerella rufomaculans* (Berk.) S. & v. S. The spice bush and particularly the sassafras are so widespread in the lower part of Delaware that they are being considered as weeds. The apple is well adapted to this soil and climate and it is rapidly gaining a high rank in the agriculture of the State. In view of these facts, it is important to exterminate both the spice bush and the sassafras and thus prevent them from harboring and carrying the bitter rot fungus to the apple.

SUMMARY

A new Gloeosporium disease of the spice bush (*Benzoin aestivale*) is recorded on both fruits and tender leaves.

The spice bush Gloeosporium is an active parasite.

The spice bush Gloeosporium is the same as *Gloeosporium officinale* E. &. E. from the sassafras (*Sassafras variifolium*) as proved by cross inoculations. Both the Gloesporium from the spice bush and *Gloeosporium officinale* appear to be the same as *Gloeosporum fructigenum* Berk. which causes the bitter rot of the apple, since each may infect both the apple and the sweet pea.

DELAWARE COLLEGE AGRICULTURAL EXPERIMENT STATION,
NEWARK, DEL.

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[3] Taubenhaus, J. J. A study of some Gloeosporiums and their relation to a sweet pea disease. Phytopathology 1: 196–202. 1911. A further study of some Gloeosporiums and their relation to a sweet pea disease. Phytopathology 2: 153–160, 1912.

THE ORIGIN AND DEVELOPMENT OF THE LAMELLAE IN COPRINUS MICACEUS

Michael Levine

The question as to the origin and method of development of the gills in the hymenomycetes still remains unsettled. Certain stages are well known and have been many times described and figured and certain general conclusions are widely accepted. For example, that in many forms the gills arise endogenously and that the relative positions of the stipe, pileus and hymenium are the same in the undifferentiated button as in the adult. On the other hand, the question as to the method of origin of the gill cavity, the direction of growth of the gill rudiments and their relation to the stipe and pileus, the differentiation of trama and hymenium, etc., have never been clearly and adequately described. The older literature has been reviewed by Atkinson (1906) and others, and I will note only such points as bear especially on my own observations on *Coprinus micaceus.*

Schmitz (1842) describes the gill cavity in *Agaricus Bulliardi* as an annular cavity separating pileus and stipe. The layer of hyphae connecting the outer margin of the pileus and stipe he called the veil or cortina. It is not clear what he means by veil as compared with the peripheral layer found in *Coprinus micaceus.* Schmitz proposed a theory of development for all pileate species of fungi according to which the organ nearest the substratum in the mature form is the structure first to arise, thus the mycelium precedes the stipe and the stipe the pileus while the hymenophore is formed last. Hoffmann's (1856) description of the development of the carpophore lies at the basis of many of the current accounts of the method of formation of the pileus and hymenium. He describes the young buttons of *Agaricus campestris* as small spheres which elongate owing to the growth of the interior cells perpendicularly upward. The terminal cells now grow out laterally and then turn abruptly downward. The ends of these hyphae form the lamellae primordia. In a later paper (1860) he described sixteen further species of Agaricaceae and still later (1861) he described the development of *Coprinus fimetarius.* Hoffmann noticed that the fungus appears at first in the form of a small white sphere in which a brown-colored central portion, the future pileus, is clearly differentiated. His description of the formation of the lamellae does not in any way differ from his previous description of other angiocarpous forms. The next work of importance that followed Hoffmann's was that of De Bary (1866), who also studied the development of *Agaricus campestris*, *A.* (*Pholiota*) *praecox* and *Coprinus micaceus*. He held that the young carpophore begins as a mass of delicate hyphae of uniform diameter densely interwoven. De Bary does not describe the growth direction of the hyphae. Very early this nodule becomes divided into two parts by the formation of a horizontal annular cavity which appears in longitudinal section as two openings in the upper and inner portion of the undifferentiated mass. The region lying above a horizontal plane through this cavity forms the pileus while that below forms the stipe. The hyphae which form the margin of the pileus are continuous with the superficial cells of the stipe. The layer of hyphae directly above the gill chamber grows into it and forms the lamellae. In *Coprinus micaceus* and *C. fimetarius* the lamellae grow into the gill chamber and in the fully developed carpophore are found in contact with the stipe. De Bary's figures of *C. micaceus* clearly show in young stages the edges of the gills in contact with the stipe, though he does not emphasize their connection with it. For *Amanita muscaria* and *A. rubescens* De Bary observed that in buttons somewhat more than 10 mm. long the hymenium and the lamellae in general are already formed and that the lower and inner margins of the lamellae are continuous with the tissue of the stipe just as the upper margins are continuous with the substance of the pileus.

R. Hartig (1874) practically accepts Hoffmann's views as to the origin of the stipe and pileus. In *Agaricus* (*Armillaria*) *melleus* he finds that the lower surface of the pileus forms an exposed hymenium. A vigorous downward growth of the hyphae now sets in from the upper surface and margin of the pileus and a corresponding upward growth of the superficial hyphae of the stipe thus forming a weft of hyphae or a veil hiding from view the hymenium. This differs from Hoffmann's view only in that the hymenium is formed superficially. De Bary (1884) ten years later was misled into accepting Hartig's view as true for a large number of further types. Atkinson (1914[3]) has recently reinvestigated this form and found that in *Armillaria mellea* as in many other species of the Agaricaceae the hymenium is endogenous in origin.

Brefeld (1877) maintained that the carpophore of the Coprini arises from a single cell. This cell is not specially differentiated and Brefeld's main interest was in maintaining that there is no morphological equivalent of the ascogonium of the Ascomycetes in the Basidiomycetes. The carpophore *anlage* is a mycelial hypha which gives rise to a number of branches which intertwine, forming a small mass of coiled hyphae. This mass increases in size and internal differentiation sets in. The whole carpophore is covered by a loose layer of globular cells which Brefeld holds is morphologically equivalent to the volva of the Amanitas and differs only in that in the latter genus the structure is more compact. Brefeld held that the lamellae arise as compact bundles of parallel hyphae, each of which has apical growth. Numerous branches are produced which turn to the right and left to form the hymenium. The growth of the lamellae results finally in the adhesion of their edges to the surface of the stipe. He found in large carpophores of *Coprinus stercorarius* that there are about one hundred and fifty lamellae of which one third are primary, two thirds secondary.

V. Fayod (1889) on the basis of studies on a long series of forms discards Hoffmann's conception of the method of development of the pileus and hymenium. Fayod held that in the upper portion of the spherical button a layer of dense hyphae is differentiated which has the form of an inverted bowl. This dense inner portion Fayod calls the *couche piléogène.* He admits that the pileus widens by lateral growth of the hyphae of this pileogenous mass.

Practically nothing further was done during the fifteen years following Fayod's work, but in 1906 Atkinson again took up the question as to the mode of differentiation of the carpophore, especially the veil and lamellae as found in *Agaricus campestris* var. *Columbia* and *A. campestris* var. *Alaska.* The gill rudiments appear first in a longitudinal median section of the young caropophore as two deeply stained areas. These areas represent the cross section of a heavily stained horizontal ring composed of hyphae which have a very dense protoplasm. This annular layer is the hymenial primordium. The hyphae below this structure rupture and an annular hollow cavity, the gill chamber, is thus formed. The lamellae are formed by a downward growth of hyphae from the hymenial primordium into the gill chamber. In (1914[1]) Atkinson confirmed his observations made on *Agaricus cam-*

pestris by a study of *A. arvensis* and *A. comtulus*. In a longitudinal median section of *A. arvensis* the first indication of any internal differentiation in the carpophore primordium is the appearance of two light areas. These represent the beginning of the annular gill chamber, thus differing from *A. campestris*. Atkinson (1914[2]) in a preliminary report on the development of *Amanitopsis vaginata* and *Lepiota clypeolaria*, holds with Fayod that the primordium of the pileus is the first to develop in the young carpophore. In *Amanitopsis vaginata* he now finds that the trama is continuous with the tissue of the pileus and the surface of the stipe. There is no internal annular gill cavity. In *Lepiota clypeolaria* as in *Armillaria mellea* (1914[3]) Atkinson describes the first appearance of the hymenium as two masses of hyphae densely stained in the upper part and on opposite sides of a longitudinal section of a young carpophore. The annular gill chamber is formed and then radial plates of hyphae from the primordium grow into the gill chamber forming the lamellae.

Miss Allen (1906), one of Atkinson's pupils, has studied a number of species of Hypholoma and establishes the endogenous origin of the gills in the several species as against the claims of Hartig for *Armillaria mellea*. Fischer (1909) studied the morphological development of *Armillaria mucida* Schrad. but adds nothing to our understanding of the origin and development of the lamellae and the hymenium.

Beer (1911) also confirms the endogenous origin of the hymenium in three species of Agaricaceae. Zeller (1914) studied the development of *Stropharia ambigua* using young buttons collected in the field. He describes the differentiation of the young carpophore as consisting first in the formation of an endogenous annular rudimentary hymenium. He claims that the pileus is formed later by an upward growth of hyphae from the outer edge of the hymenium primordium so as to form an internal inverted cup-shaped structure below the upper portion of the carpophore. The gill chamber is formed by the sagging of the tissue below the hymenium primordium. The lamellae are formed by the downward growth of radial hyphae. The apical cells of these hyphae spread laterally thus forming a groove along the edge of the gill. The hymenial hyphae arise as branches near clamp connections in the subhymenium but gives no figures in support of his statement. Zeller believes that the formation of the hymenium in all annulate agarics precedes that of the pileus primordium while in other agarics the pileus primordium develops first. Zeller does not make clear what becomes of the upper portion of the young carpophore in the annulate agarics which is present before the development of the hymenium when the so-called pileus primordium is formed.

In studying the nuclear phenomena in the mycelia of a number of pileate fungi I found that *Coprinus micaceus* grows well in culture media and is favorable material for studying the formation of the various structures of the carpophore, particularly the lamellae.

Methods

The carpophores of *Coprinus micaceus* were collected in great numbers during the summer of 1913 in the vicinity of New York. Petri dishes thoroughly cleaned and partly filled with water were covered with circular pieces of oak tag in which two to three circular openings were made somewhat smaller than the diameters of the pilei. The caps were placed over these openings and the Petri dishes were covered. After six to ten hours when the spores had been sown the caps were removed and the pieces of oak tag covered with spores were likewise taken from the dishes and carefully folded and preserved. In tap water germination occurs in about twenty-four hours. The germinating spores were transferred to pint milk bottles containing various media (*a*) soil in which the plants had been growing; (*b*) soil mixed with horse dung; (*c*) horse dung; (*d*) pieces of wood taken from the same soil; (*e*) beef agar; (*f*) carrot; (*g*) beans; (*h*) beet leaves; (*i*) string beans; (*j*) horse dung agar; (*k*) corn meal agar. From forty to sixty days after inoculation young carpophores appeared on the soil, dung and cornmeal agar media. In cases where the medium became too dry sterilized water was carefully added by means of a pipette.

Buttons were carefully removed from the cultures and fixed. The substratum was teased away and the young carpophores were transferred to vials. In case of the agar media the agar was cut away and buttons and medium together were put in a fixing bath. A number of fixing solutions were used, Bouin's, Merkel's, picro-acetic, and chrom-acetic. The best results were obtained, however, with Flemming's weaker mixture. After twelve to twenty-four hours the material was washed in running water, carefully dehydrated and imbedded in 52° paraffin. Sections 5 μ to 7.5 μ in thickness were made. The preparations were stained with iron haematoxylin, eosin, acid fuchsin, and congo red, but principally with Flemming's triple stain.

The Carpophore Primordium.

The facts as to the development of the carpophore which may be regarded as settled are as follows: 1st. The relative positions of the stipe, pileus and hymenium are essentially the same in the undifferentiated button as in the adult. 2d. The endogenous development of the hymenium as shown by Hoffmann, Brefeld, Fayod, Atkinson and others.

The points on which further light is needed are: (1) Does the carpophore originate from a single hypha? (2) The origin and direction of growth of the hyphae that form the pileus. (3) The origin and direction of growth of the hyphae that form the lamellae, especially the hymenium. (4) The method of origin of the gill chambers.

I shall use the terms pileus primordium and pileus fundament as corresponding to the *couche piléogène* of Fayod. As I have noted, carpophores of *Coprinus micaceus* are developed in cultures containing cornmeal agar, soil, horse dung, etc., in from eight to nine weeks. A sparse mycelium of white hyphae appears over the surface of the substratum in a week. After six to eight weeks dark brown rhizomorphs appear growing upon the inner surface of the glass container. In about two or three weeks more small white or slightly grayish buttons may be expected to appear on the rhizomorphs on the glass, below the level of the medium and on the medium itself. After a day or two the buttons expand into the carpophores. Such media as cooked carrot, crushed beet leaves, beans, string beans, or bread soaked in grape decoction produce a dense mycelium but neither rhizomorphs nor carpophores are formed so far as my observation goes. Cornmeal agar cultures in test tubes last longer than those in milk bottles. The moisture is better conserved and the mycelium forms a thick layer over the agar upon which carpophores are formed. Such cultures may be studied directly with the low powers of the microscope. The young buttons develop both on mycelial strands in the agar and on the rhizomorphs. Carpophores from both soil-dung and cornmeal agar media were fixed and sectioned.

I have not been able to determine whether Brefeld is right in claiming that the carpophores of Coprinus take their origin from a single hypha. A longitudinal median section of the youngest carpophore I have studied is shown in *figure* 1. The young button consists of a tuft of hyphae whose direction of growth is markedly upward and somewhat divergent, as figured by Brefeld (1877), for *Coprinus* *stercorarius* and rests on the surface of a compact layer of hyphae which covers the agar. The mycelial layer covering the agar consists of a dense plectenchyma of septate hyphae, the walls of which are gelatinous and stain heavily. The hyphae in contact with the agar are abundantly branched and extend down into it forming a very loose network. These hyphae have thin cell walls and a fine granular protoplasm with nuclei easily differentiated in staining. The appearance of these young buttons suggests that they originate by the upgrowth of numerous hyphae from the mycelium covering the agar. That portion of the core of the button nearest the substratum is very compact and the hyphae here appear to be more abundantly branched. The upper portion of the button consists of a loose, well aerated structure of more or less intertwined hyphae. The hemispherical pads, shown by Strasburger (1884) as marking the presence of the so-called protoplasmic pit connections between hyphal cells are conspicuous. The nuclei in all of these cells are well differentiated by Flemming's triple stain. The nucleoles are ruby red while the chromatin is blue.

The surface of the button consists of a fairly well marked zone of rather straight radial hyphae which arise from the outer cells of the mass. Near the substratum these radial hyphae are short and almost horizontal, at the apex of the button they are vertical. These hyphae branch and make a rather uniform peripheral layer over the central region. Each hyphal filament (*fig.* 2) in the layer is about six to eight cells long and one or two of the outer or terminal cells are slightly larger and spherical or oval in shape and their walls are thick and gelatinous. Brefeld's (1877) account of the peripheral layer of the young carpophore of *Coprinus stercorarius* applies perfectly to that of *Coprinus micaceus.*

PILEUS PRIMORDIUM

A little above the central region of the button and just below the peripheral layer an accelerated growth of intertwining hyphae results in the formation of a denser mass which is the beginning of the pileus. The hyphae forming this mass are narrow, much branched and with dense easily stained cytoplasm. This pileus primordium can be distinguished before the sections are stained by its density as contrasted with the looser middle portion below and the thick walled cells of the peripheral layers. It is very small at first but apparently by the rapid growth and branching of its hyphae it broadens out and soon suggests the outlines of the future cap (*fig.* 3) as shown by Fayod (1889) in a number of agarics. Its development is very regular and results in a hemispherical mass of compact narrow interwoven hyphae. At the same time there is a vertical elongation of the carpophore primordium. In the region immediately below the young pileus this vertical growth leads to the formation of parallel strands of hyphae which become the stipe. The cells of the hyphae in this portion of the carpophore appear to have grown especially in their vertical diameters. Their cytoplasm is not dense but is evenly distributed and stains very faintly in comparison with that of the hyphae in the young pileus. In the basal portion of the young carpophore the hyphae are more or less twisted upon each other and their cells are much shorter. Their cytoplasm is somewhat denser and the nuclei can be more easily differentiated. *Figure* 6 shows the appearance of the hyphae which lie in the mycelium and in the lower portion of the carpophore. These cells are long, and their walls are thick and gelatinous. Their cytoplasm contains large and irregularly scattered vacuoles. The nuclei show clear ruby stained nucleoles and finely granular chromatin. The formation of the hemispherical pileus primordium as Fayod has pointed out divides the young carpophore into three distinct regions, the peripheral layer, the pileus primordium and the tissue which will subsequently form the stipe.

The Hymenium

In a slightly older stage we find the beginning of the differentiation of the hymenium. The development of the hymenial elements seems to begin at or near the lower surface of the densely interwoven mass of hyphae which is to develop into the pileus. The whole carpophore is still oval in outline. There is as yet no indication of narrowing to form a stipe. In vertical section the first indication of the developing gills consists of two dense areas symmetrically placed to the right and left above the center of the carpophore as has been so frequently shown in recent papers. They are distinctly endogenous in origin and lie about one quarter of the distance from the surface to the center of the carpophore. Careful study shows that the hyphal cells in these regions are forming downwardly arched ridges of densely staining palisade cells. These palisade cells it may be noted arise in quite a different fashion than that described by Fischer (1909) for *Armillaria mucida* Schrad. and Beer (1911) for *Clitocybe laccatus.* These authors hold that the hymenium is formed by an inward extension of the subcuticular palisade cells which cover the pileus. In *Coprinus micaceus* we have from the very beginning a series of radially placed ridges consisting of a palisade of hyphal cells. The palisade cells observed here appear long before the so-called subcuticular layer of the pileus is differentiated. These young gill cells are rather long, their walls are thickened and stain somewhat more deeply than those of the cells of the pileus primordium (*fig.* 7). It is not easy to determine whether these palisade cells of the young gills become the basidia directly. It is quite probable that further apical growth and branching lead to the definitive formation of the hymenium.

Figure 8 represents a stage a trifle later. The figure shows a portion of a tangential section of a young button which is still cylindrical or spherical or oblong in shape. The growth of the hymenial palisade layer is toward the surface and progresses proportionately to the increasing width of the primordium of the pileus. At the same time the length of the ridges increases by the formation of new palisade cells from above. It is very clear in this stage that the palisade cells do not enclose the edge of a gill but enclose the notch between two gills, that is, they form in section a V opening doward and not upward. Where the ends of the first formed palisade cells meet a small opening appears representing the beginning of a gill chamber of which a somewhat later stage is shown in *figure* 9. The further development of palisade cells leads to the still further enlargment of the gill chamber. A longitudinal median section of a young gill chamber at this stage can hardly be made thin enough to show these relations clearly. Cross-sections of the forming gills are much more instructive. The tangential section shown in *figure* 8 is in a slightly older stage. Here we have a series of newly formed gill chambers. It is obvious at once that the spaces between the gills are not connected to form a single continuous annular cleft as described by Hoffmann, Atkinson and others. In reality we have here a series of radial gill chambers, one between each pair of lamellae. The hyphae of the trama of each gill run straight through into the stipe below and the pileus above. *Figure* 9 represents the young lamellae with the gill chambers between them on a large scale, showing the connections of the hyphae of the trama. The gill chamber is not altogether empty for some of the hyphae from the stipe seem to branch and grow into it as shown by Atkinson (1914[3]) for *Armillaria mellea*. *Figures* 10, 11, 12 represent hyphae from the trama and stipe in a somewhat later stage; two nuclei are found in each cell. The character of the branching and the continuity of the stipe and gill hyphae are clearly shown.

We have then in the differentiation of the pileus and the upper portion of the stipe region of *Coprinus micaceus* first the formation of a palisade layer of ridges of cells for each pair of hymenial surfaces which line the cavity between two adjacent gills. The gills are strictly endogenous in origin. There is no general annular gill cavity as described by Hoffmann, De Bary, Atkinson, and others, and no annular hymenial primordium.

As the horizontal diameter of the carpophore increases we find that the differentiation of the pileus and lamellae progresses centrifugally. As the growth progresses the distances between the lamellae primordia increase and new gill primordia arise between the old ones as shown in *figure* 13.

Further Development of Pileus and Stipe

The peripheral layer of the pileus which was originally composed of cylindrical hyphae capped by one or more oval cells now becomes modified. The cylindrical cells of the earlier stages become oval. Stages shown in *figures* 8, 13 represent later stages in the development of the pileus. The peripheral layer is now made up of a few cylindrical cells nearly all of which are globular and gelatinous. The change in form takes place from the outer toward the inner cells as Brefeld (1877) described. The thickness of the layer is greater over the apex of the pileus and decreases as it approaches the base of the young carpophore. The structure of the pileus proper has undergone a marked change. The fine compactly coiled hyphae seen in the earliest stages have become much less compact so that the individual cells are more distinct. The hyphae widen and the spaces between them become larger. They are nevertheless still intertwined and show no definite direction of growth. In the lower central portion of the pileus which lies in close proximity to the stipe region the hyphae also interlace yet many may be traced directly into the stipe below while others may be traced directly upward to the periphery of the pileus. The hyphae near the upper surface of the pileus give rise to short, stubby, vertical branches consisting of a longer terminal cell and one or two shorter ones below. These branches lie parallel to each other and form a palisade layer over the pileus (*fig.* 15) below the outer peripheral layer. This structure is similar to that described by Fayod (1889) and later by Fischer (1909).

In many cases the nuclei in these cells are well differentiated and both the nucleoles and chromatin granules are visible. The hyphae found in the portion of the pileus over the lamellae are oriented and appear to be growing downward; still in many well stained sections hyphae are also found growing downward as indicated by the direction of the branching. In somewhat later stages the flesh of the pileus is made up of broad septate hyphae having a sparse granular cytoplasm in which two nuclei are regularly present. The lamellae are still continuous with the tissue of the stipe below just as they are with the pileus above. The hymenial surface is sharply differentiated and distinct basidia and cystidia are recognizable. There are no basidia formed on the edges of the gills, as shown by Brefeld for other species of Coprini.

Figure 8, a longitudinal tangential section very close to the median, represents best the structure of the stipe at this stage. The basal portion consists of a compact intertwining mass of hyphae (*fig.* 5) from which the hyphae of the center of the young stipe arise. The hyphae in the stipe which lie exterior to the central region are still continuous with the hyphae in the young gills. As we approach the outer layer of the stipe, that is, the layer just below the peripheral layer of globular cells we find the hyphae become narrow. The density of the cytoplasm and the thickness of the cell walls make this a well differentiated layer. These hyphae can be traced into the pileus where they are continuous with the cells of the surface there. The growth direction is upward as shown by the character of the branching. The further development of the stipe consists primarily in the vertical elongation and swelling of the hyphae in the outer and middle layer of the stipe. A cross section of the young stipe shows that there are two types of hyphae, the wide and the narrow, similar to those which have been described by Harper (1902) for *Coprinus ephemerus*, although at this stage the broad type predominates. The cells in these hyphae are long as compared to their width and have a homogeneous cytoplasm in which a large number of nuclei are found (*fig.* 16). The nuclei are clustered and lie in the center of the cell.

The increase in size of the stipe is accompanied by the increase in size of the primordia of the lamellae and an outward and downward growth and expansion of the pileus. The hyphae in the upper portion of the stipe near the pileus are intertwining and septate and two or three nuclei are invariably found in each cell as shown in *figure* 18.

The significance of this structure is not clear, although it may be the point of origin of secondary growth in the pileus as suggested by Miss Allen (1906).

SUMMARY

1. The carpophore primordium of *Coprinus micaceus* arises from the mycelium directly or from a rhizomorph. The young button makes its appearance from forty to sixty days after either cornmeal agar or soil media are inoculated with spores.

2. The pileus initial appears as a hemispherical mass of fine, narrow, interlacing septate hyphae having a dense cytoplasm which stains heavily with Flemming's triple. The peripheral layer of cells of the carpophore primordium is differentiated very early in that they stain more deeply.

3. The primordium of the hymenium arises at or near the lower surface of the pileus primordium and appears in vertical section as two densely stained areas of palisade cells, symmetrically placed to the left and right above the center of the carpophore primordium.

4. A palisade of hyphal cells is formed pointing obliquely downward so as to form a series of arched ridges. These ridges form the young gills. The hymenial elements do not enclose the edge of a gill but enclose the notch between two gills.

5. The small opening formed at the point where the first formed palisade cells meet is the beginning of a gill chamber. The further development of palisade cells leads to the enlargement and further development of the gill chamber.

6. Longitudinal tangential sections show the gill chambers in series, one gill chamber between each pair of lamellae. The hyphae of the trama of each gill run straight through into the stipe below and the pileus above.

I wish to express my deep appreciation to Professor R. A. Harper for his many helpful suggestions. I also wish to thank Professor W. G. Marquette for his assistance in making the microphotographs.

COLUMBIA UNIVERSITY,
NEW YORK CITY.

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EXPLANATION OF PLATES XXXIX AND XL

The figures in Plate XL were drawn with the aid of the camera lucida from preparations stained with Flemming's triple stain. Leitz 1/16 objective and No. 1 ocular were used in making figures 9 and 15; the rest of the figures on this plate were made with the 1/16 objective and No. 3 ocular. Distance from camera lucida to drawing board 90 mm. Microphotograph 1 was made with Leitz objective No. 6 and ocular No. 3; 3, 8, 13 and 17 were made with objective No. 3 and ocular No. 3.

Cramer's micro-ray filter No. 7 was used.

PLATE XXXIX.

FIG. 1. Vertical median section of an undifferentiated carpophore, on agar. × 250.

FIG. 3. Vertical median section of young carpophore in which the pileus primordium is differentiated, on agar. × 170.

FIG. 8. Vertical tangential section showing arched palisade tissue and a series of gill chambers. × 285.

FIG. 13. Vertical tangential section of a somewhat older button showing a series of gills with separate and independent gill chambers between them. Secondary gills alternating with the primary gills. × 85.

FIG. 14. Portion of figure 13 enlarged, showing (*a*) primary gills, (*b*) gill chamber, (*c*) pileus, (*d*) secondary gills, (*e*) hymenium, (*s*) stipe.

FIG. 17. Section of a stipe in a well-differentiated carpophore, showing nuclei clustered in the center of the cell. × 120.

PLATE XL.

FIG. 2. Hypha from the peripheral layer in the young carpophore.

FIG. 4. Hyphae from the basal portion of the young carpophore.

FIG. 5. Same as figure 4, but later stage.

FIG. 6. Hypha from the mycelium near base of young carpophore.

FIG. 7. Part of a vertical tangential section of a young carpophore showing ridges of palisade cells which form gill primorida.

FIG. 9. Part of a vertical tangential section of a carpophore somewhat older showing the palisade cells of the hymenium and two of a series of gill chambers. The hyphae of the trama are continuous with the hyphae of the stipe below and the pileus above.

FIG. 10. Hyphae from the trama showing branching upward.

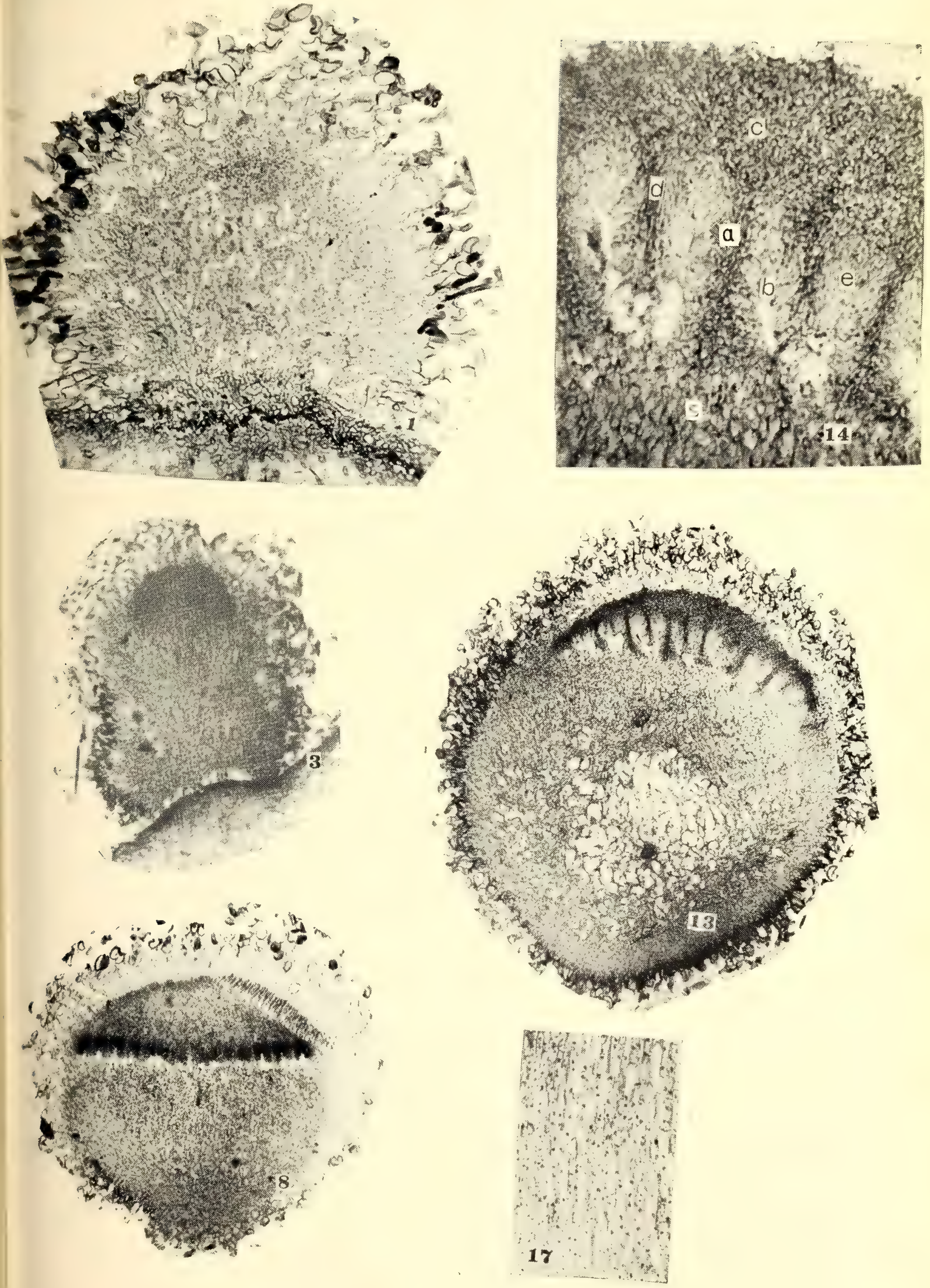
FIG. 11. Other cells from the trama.

FIG. 12. Hyphae from the stipe which connect above with the hyphae of the trama such as are shown in figures 10 and 11.

FIG. 15. Part of the upper portion of the pileus showing the palisade arrangement of the cells below the peripheral layer.

FIG. 16. Cells from the portion of the stipe shown in figure 17, enlarged, showing numerous nuclei.

FIG. 18. Hyphae from the upper portion of the stipe near the pileus in a well differentiated carpophore.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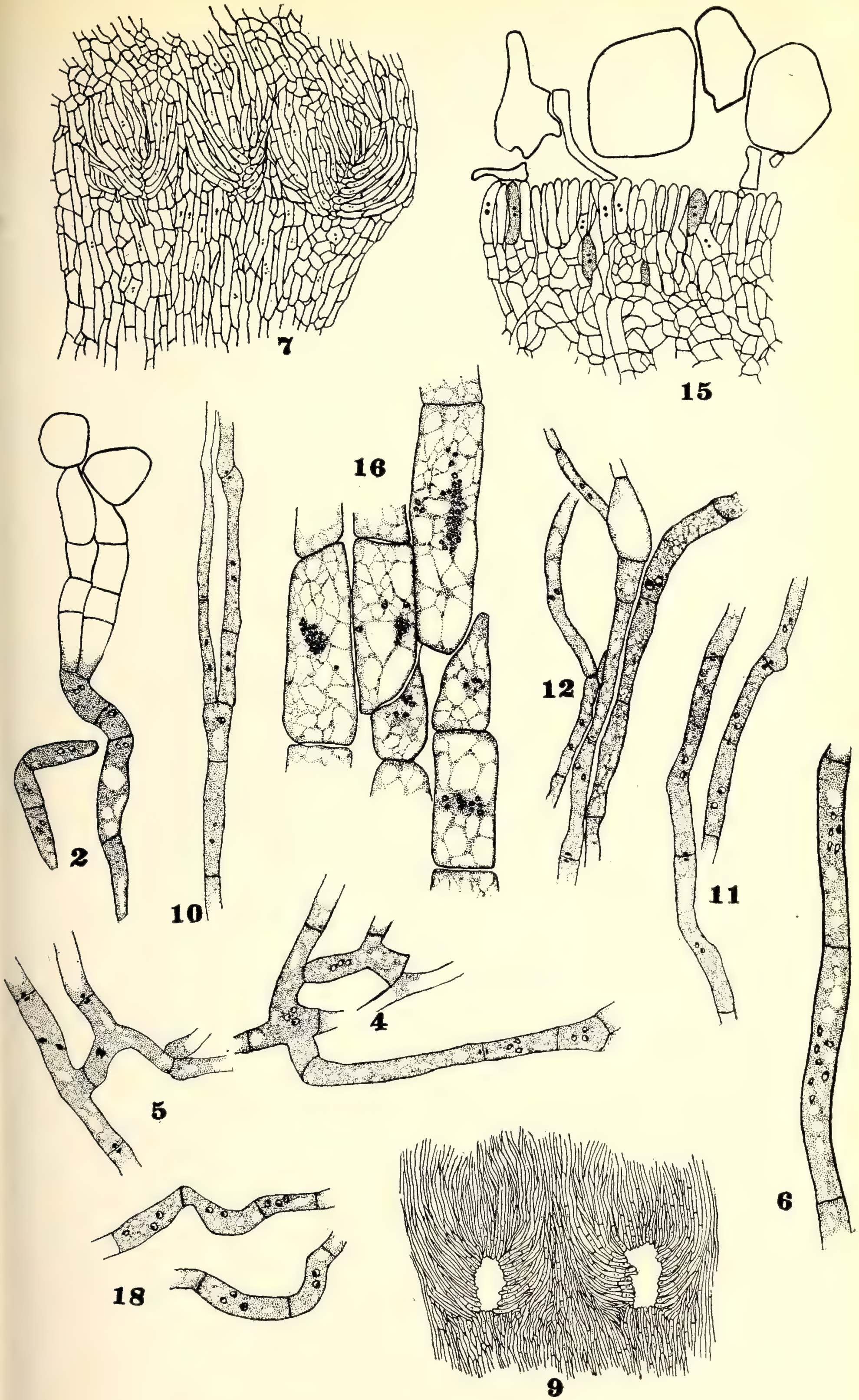
Levine: Origin of lamellæ in Coprinus micaceus.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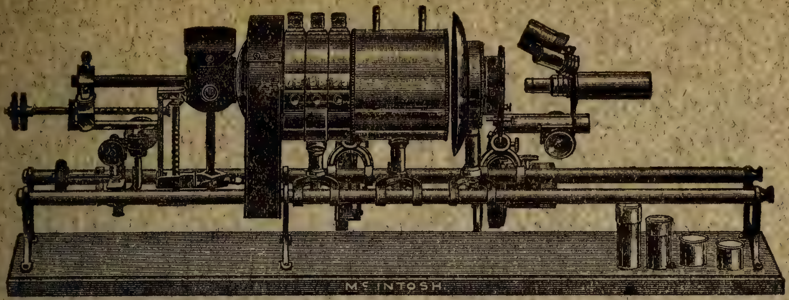

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STUDIES OF THE DEVELOPMENT OF THE PIPERACEAE

II. The Structure and Seed-Development of Peperomia hispidula

(Continued from page 339 of this volume)

Duncan S. Johnson

D. The Carpel and Fruit; the Ovule and Seed

The development of the ovary of this *Peperomia* is initiated by a swelling out of the base of the floral bract, due to the elongation of the subepidermal cells of the upper side of its stalk (figs. 24, 25, 26). This elevation, convex at first, later becomes flat, and then concave, as the rapidly growing margin pushes upward to form the wall of the single celled ovary (figs. 53, 54). Before this wall closes together to form the stigma, the floor of the ovarian cavity has begun to swell upward to form the single erect ovule, in the midst of which the primary archesporial cell has now become evident (figs. 54, 55). The carpellary tube closes in to form the stylar canal at about the time the parietal cell is separated from the embryo-sac mother-cell (fig. 56). It is noteworthy that, from early in its development, the abaxial margin of the upgrowing carpel is longer than that next the axis (figs. 55, 56). This longer side ultimately gives rise, at its tip, to the papillose, rounded, simple stigma (figs. 56, 57, 78).

The wall of the carpel is at first only three or four cells in thickness (fig. 55). The portion about the ovule increases but little in thickness thereafter, and in the mature fruit is only 4 or 5 cells thick, except in one longitudinal strip along the abaxial side. There, in the mature fruit, it may become 6 or 7 cells in thickness (figs. 78, 100, 101, 108). In the stylar region, however, periclinal walls appear more abundantly, especially on the abaxial side, and a section of the style may show it to be 16 or 18 cells in diameter (figs. 78, 79). Of the 4 to 7 layers of cells

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making up the wall of the ovary, only the innermost and outermost are appreciably specialized in structure. The only exception to this is the single delicate strand of vascular tissue running down the abaxial side (figs. 100, 101, 108). This strand is well-developed by the time the embryo sac is mature (fig. 78). It consists from this time on of but 2 or 3 ringed ducts, 3 or 4 μ in diameter, and of a few small, elongated cells nearby, that may play the part of phloem (figs. 100, 101, 108.) This simple vascular bundle connects with the lattice-like tracheids of the style above, and with the vascular bundle of the stalk of the fruit below. Aside from these vascular tissues, the 2 to 5 cell-layers of the carpel between its inner and outer layers, consist of but slightly elongated, thin-walled parenchymatous cells (figs. 101, 108).

Up to a late stage in the development of the fruit, the cells forming the innermost layer of the carpel remain thin-walled, and each may contain two or three dozen chloroplasts. In the mature fruit, however, the inner walls of these cells and the inner portions of all four radial walls become slightly thickened, and the whole cell contents turn brown and shrink against the inner walls of the cell (fig. 108). The chloroplasts of these cells, though shrunken and brown, do not lose their identity, as they do in the dried contents of these same cells in *Peperomia pellucida* (Johnson, 1900*a*, fig. 15). Neither are the walls of these cells thickened to the same degree, nor in the complex lattice-like pattern of those of *P. pellucida.*

The outer epidermal layer of the carpel is far more highly specialized than any other. It consists, when mature, of four distinct types of cells, the oil-cells, the hydathodes, the bristle-bearing cells and the ordinary less modified epidermal ones. The latter differ in size and shape (figs. 107, 108). Their convex outer walls are slightly thickened with a corrugated cuticle, like that of the epidermis of the stem and leaf (cf. fig. 13). The oil-cells are structurally the least modified of the other surface cells of the fruit. They are nearly isodimensional, and are commonly shaped like truncated pyramids with their bases inward (figs. 78, 100, 107, 108*a*). Up to the time of the maturing of the embryo sac in an ovule, the protoplast in each of the oil-cells of its carpel is thick and the nucleus and vacuole about half the diameter of the cell. In the mature fruit the protoplast is usually somewhat shrunken and, in unstained material, only a few small yellowish granules are visible. Imbedded in the part of the protoplast lying against the outer wall, however, is a disk of a substance that stains a dense black with iron haematoxylin (figs. 100, 108, 108*a*).

The glandular hairs, or hydathodes, as they probably are in function, occur only on the stalk, style, and adjoining base and tip of the ovary (figs. 78, 100, 108). The structure of these hydathodes is the same as that of those occurring on the leaf. With the ripening of the fruit the hydathodes may shrivel somewhat and turn brown, and the outer cells may sometimes drop off (fig. 108).

The most highly specialized of the cells of the surface of the fruit are the tapering, unicellular bristles. These project radially from all sides of the swollen fertile portion of the ovary, and are found nowhere else on the plant (fig. 107). Each bristle is initiated by the protuberance of one of the larger surface cells of the carpel, which occurs at about the time the endosperm nucleus is being formed by fusion (fig. 101). The cell continues to elongate until it attains a long conical form with a diameter of 8 or 10 μ at the base, and a length of 300 or 400 μ. The mature bristle has from 2 to 6 longitudinal ribs along the inner surface of its wall, each of which may become 2 or 3 μ in thickness and 5 μ wide (figs. 108, 109). At the base of the bristle these ribs run down to the inner wall of the epidermal cells, and there may be bent outward, thus bracing the bristle more effectually (fig. 108). These bristles remain intact, stiff and rigid, on all the mature fruits seen. It is therefore, possible that they may be effective in keeping snails and small insects off the fruits, but no conclusive evidence has been obtained on this point. It is of interest to note the relatively late appearance of these bristles, as compared with that of the multicellular hairs of the leaf, which appear while the latter is still very young.

Besides the middle, fertile portion of the ovary, with which we have thus far been concerned, there are certain interesting structures to be noted in the basal stalk and the terminal style and stigma. The stalk of the ovary and of the fruit, though not as long as in species like *P. obtusifolia*, is still quite distinct (figs. 78, 100, 107). It has a length, from the chalaza to its insertion on the axis, of about half a millimeter. Its diameter is about .2 of a millimeter. The epidermis of the stalk is like that of the body of the fruit, except that bristles are absent and hydathodes are abundant. The most interesting feature of the internal structure of the stalk is the vascular tissue. For two thirds the way downward from the chalaza there are two distinct groups of these cells. One group is central or axial; the other, coming down from the abaxial side of the body of the carpel, does not fuse with the proper bundle of the stalk until the base of the stalk is nearly reached (figs. 100, 108). The central vascular strand consists of but 3 or 4 xylem elements, and perhaps a few phloem elements. These are embedded in a mass of parenchyma. The central bundle ends above, with a considerable number of bent and spiral tracheids, just below the thickened cells of the chalaza (fig. 108). The walls of these latter cells begin to thicken before the fruit has attained its mature size. It seems evident that food material brought up by the bundle and the parenchyma of the stalk must be able to pass through these partially thickened cells of the chalaza, for the walls of the integument, the only other possible path to the ovule from without, are at this time, still more greatly thickened. The stalk, as has been stated above, finally ruptures at the constricted portion of the base, and thus sets the ripe fruit free. At this constriction the stalk is abruptly narrowed to half the diameter, it has immediately above, and the epidermal cells are very thin-walled. Both these features of the structure make the break easier, and strictly localize it (figs. 100, 107, 108).

The style and stigma are developed by the continued growth of the upper margin of the carpellary ring, after the latter has closed together above the ovule to form the distinct stylar canal (figs. 56, 57). The stigma is formed solely from the abaxial and longer lip of this upgrowing carpel (figs. 78, 100). It consists, when ready for pollination, of a knob or rounded end made up of parenchyma, and covered by a layer of papilla-tipped columnar cells. These latter have large nuclei and dense cytoplasts, and form the surface for the attachment of pollen grains (fig. 78). With the initiation of the endosperm the mature stigma shrivels and remains on the ripe fruit as a cap of black cells at the end of the style (fig. 107). No germinated pollen grains were detected on the stigma.

The style of this plant which is remarkably long for a *Peperomia*, is marked off from the stigma by a definite constriction (fig. 78). From this point down to the shoulder of the fruit the style is 180 to 200 μ long and about 150 μ in diameter. The ordinary epidermal cells are interspersed with oil cells and hydathodes, but no bristles (figs. 100, 107, 108). Internally the style consists of more or less elongated cells, which are at first much alike (fig. 57). Later longitudinal walls, in the neighborhood of the stylar canal, give rise there to a small-celled tissue, with dense protoplasts, which evidently serves as a conducting tissue for the pollen tubes (figs. 78, 100). At least, the only structures seen that resembled pollen tubes were growing through this tissue. Latticed tracheids, to the number of 10 or 12, and with a diameter of from 10 to 25 μ, arise in the tissue of the abaxial side of the style. These tracheids form the expanded upper end of the slender vascular strand that runs along this side of the fruit (figs. 78, 100, 107). The position and structure of these tracheids suggests that they may serve for water storage, perhaps to prevent premature shriveling of the style and stigma. These cells, however, do not reach their highest development till some time after pollination.

We may now take up the details of development of the ovule, with its single integument, into the seed with its seed coat.

The nucellus of the ovule is initiated by the upward stretching of the subepidermal cells at the center of the carpellary ring (figs. 53, 54). The mound so formed continues its growth until, by the time the carpel has closed together above, it forms a cone of about 30 cells in vertical section (fig. 56). The growth of the nucellus continues until, by the time the egg is differentiated, it is 10 to 15 cells broad and 25 to 30 cells long (fig. 78). The cells of the peripheral layer are somewhat flattened radially, while in the interior of the ovule the short diameter of the cells at this stage is the longitudinal one. The group of cells above the sac, formed by the division of the parietal cell, consists at this stage of 8 or 10 cells that show in a longitudinal section (figs. 81, 89). In ovules that do not give rise to embryo and endosperm, growth of the nucellus stops at the stage just indicated. Many ovules that have reached this stage and then shrivelled up are found on spikes bearing ripe, or nearly ripe fruits. This shriveled condition is probably due to the lack of fertilization of the eggs in these ovules. It is true that fruits are found in which degeneration set in at an earlier, or at a later stage of development, but in by far the larger number of the ovules that degenerate, this process seems to begin after the embryo sac is mature and ready for fertilization.

In those seeds where embryo and endosperm are formed, a very marked renewal of growth of the nucellus occurs. This growth is due only in small part to increase in the number of cells present at the beginning of embryo-formation, but chiefly to an increase in the size of these cells (figs. 78, 100, 108, 111). The nucellus increases from 125 to 900 μ in length, and from 90 to 590 μ in diameter. The highly vacuolated cells seen in the nucellus in the earlier stage become the starch-filled perisperm cells of the ripe seed (figs. 101, 108, 111). The starch grains of the perisperm cells are, as in the seeds of *P. pellucida* and other species, grouped in the several distinct vacuoles of each cell, to the number of often 1,200 or 1,500 in a single vacuole. The nucleus of the ripe perisperm cell is usually squeezed up in a small central or lateral portion of the cytoplasm between the vacuoles. There is sometimes present also a large rounded body, staining slightly, which, like that found in *Peperomia pellucida* (Johnson 1900*a*, fig. 15), is probably to be regarded as a leucoplast. The cells of the outer layer of the nucellus, including those above the embryo sac, have much less starch than the central cells. In the ripe seed the starch has entirely disappeared from several layers of the cells next the embryo sac. The walls of the cells nearest the sac have completely collapsed (figs. 89, 108). This perisperm, with its starch, is absorbed through the endosperm at the time of germination, as in *P. pellucida*, which will be shown later (fig. 111).

The integument is initiated soon after the carpel closes in above the ovule, chiefly by the activity of the epidermal cells of the base of the young ovule (figs. 56, 57). This ring-like outgrowth continues pushing upward as the nucellus elongates and closes together above to form the micropyle, before the time when the embryo sac has reached the sixteen nucleate stage (figs. 74, 78). The integument is two-layered at first, and most of it remains so (figs. 68, 78, 100). At the base of the ovule and occasionally near the micropyle, it may become three-layered as the fruit approaches maturity (fig. 108). The cells of the outer layer of the integument never thicken appreciably on the sides of the fruit, but collapse and are finally crushed between the inner layer of the integument and the inner layer of the carpel (figs. 101, 108). At the base and the apex of the ovule one or both of the outer layers of the integument may be thickened like the inner one (figs. 100, 108). This inner layer consists of cells which are thin radially aud isodimensional in the other two directions (figs. 100, 101). The nuclei remain large and active until a late stage of development. The inner and outer walls attain a thickness of .5 μ or .7 μ, and have a dark brownish color in unstained material. Near the micropyle the inner walls of these cells are thrown into strong folds and protuberances, giving them a very irregular outline in section (figs. 89, 100, 108, cf. Johnson 1900*a*, fig. 15; 1910, fig. 68). The nuclei of these cells are large and active at first, but later these and the other cell contents become distorted and shriveled. This layer is clearly the chief protective layer of the seed. Near the base of the ovule the cells of the two or three layers of the integument may become thickened and brown (fig. 108). Across the base of the nucellus a series of 5 or 6 layers of transversely expanded cells become thickened, and thus serve as a protection against desiccation of the seed on this side (figs. 100, 108). The walls of these cells become brown, but are not as greatly thickened as those of the integument itsself.

It is clear that taken all together the structure of the carpel and seed coat of this *Peperomia* is much less highly specialized as a protection against desiccation than that of these same structures in *Peperomia pellucida*. In fact all other species of *Peperomia* examined by the writer show greater thickening of these tissues than is seen in *Peperomia hispidula*. It therefore seems altogether probable that the present character of these structures in the latter species has arisen secondarily, and is related to the character of its habitat.

The vascular system of the carpel, with its one slender bundle, is possibly to be regarded as secondarily derived from types like the *Pipers*, with six longitudinal vascular bundles in the carpel (Johnson, 1902, fig. 15). It must be recalled that the development of the ovary of *Peperomia hispidula* gives no hint that it has arisen by the coalescence of three carpels, which does seem true of most species of *Piper* that have been studied.

E. THE EMBRYO SAC, EMBRYO AND ENDOSPERM

1. *The archesporium and tapetum.*

Of the group of hypodermal cells visible in the young ovule from the beginning, one axial cell becomes distinguishable, about the time the carpel is closing together, by its larger size and larger nucleus. This cell divides at about the time the integuments are initiated, to form a parietal or tapetal cell above and the definitive archesporial cell toward the chalaza (figs. 56, 57). In a few ovules two such archesporial cells were seen (figs. 113, 114), which as we shall see may often go on in their development and form two embryo sacs (fig. 120). No mitoses of the nucleus of the primary archesporial cell were seen, but from mitoses occurring just before and after this stage there can be no doubt that this mitosis is a normal vegetative one in which more than 20 chromosomes are concerned.

The tapetal or parietal cell early divides by 1 or 2 periclines and two or three series of anticlines to form the 2 or 3 layers of parietal tissue found in the young seed above the sac (figs. 74, 77, 81, 89; also Johnson, 1907, fig. 2). As the seed ripens the walls of these cells become but slightly thickened. The cells of the layer next the integument often divide by a few more anticlines and elongate radially to form a series of columnar cells just below the micropyle (fig. 104). In the ripe seed the layer of parietal cells next the embryo has been crushed by its growth, the remaining layers persist intact and plump, but contrast with the perisperm cells in having few starch grains in them. At germination these parietal cells are pushed off, along with the style of the fruit, by the swelling of the endosperm as it bursts the seedcoats (see p. 375; fig. 111).

The definitive archesporial cell, or embryo sac mother-cell, is at the time of its formation nearly cubical in shape. It is about 20 μ long and 16 μ wide. Its nucleus when first organized is about 10 μ in diameter, has a distinct wall, a prominent nucleolus and a rather close chromatin net just within the wall (fig. 58). As development proceeds this embryo sac mother-cell grows, until, when ready for the next nuclear division, it may be 30-45 μ long and 25-30 μ broad. The nucleus itself continues to grow, up to and even after the time of synapsis. During the early phases of synapsis the nucleolus may have a diameter of 3 or 4 μ. Just before synapsis sets in the chromatin of the nucleus lies near the periphery in the form of a net, the threads of which have a diameter of .3 or .4 μ and form meshes 1 to 3 μ across (figs. 58, 59). The cytoplasm up to this time retains a uniform structure, with rather evenly distributed small vacuoles.

2. *The megaspore and embryo sac.*

The development of the embryo sac from the definitive archesporial cell is initiated by the occurrence of a genuine synapsis. The first evidences of the beginning of this process are seen in the increase in thickness of the chromatin threads of the reticulum, in the reduction in the number of meshes and in the gradual shrinking away of the chromatin from most of the nuclear wall. Later the whole chromatin thread forms but 1 or 2 close tangles of contracted loops near one side of the nuclear cavity (figs. 59, 60, 61). The examples of this stage seen do not give evidence of a doubling or splitting of the chromatin thread, before the compact knot stage is reached (figs. 60, 61). On emerging from the characteristic tight knot the chromatin takes the form of a rather loosely looped spireme, with distinct chromomeres scattered along it (fig. 62). These chromomeres are about half a mu in diameter and occur at intervals of 1.5 or 2 μ along the thread. The intervening portions of the thread are slightly narrower and stain much less deeply. The thread at this stage is evidently double (figs. 62, 120-19. 2-30. 6). Soon after this spireme appears it begins to segment, by constriction, to form looped and twisted chromosomes (fig. 63). These chromosomes are at first 15 or 20 times as long as thick but later they become considerably thickened or may be nearly globular in form (figs. 65, 66).

The nucleolus retains its original size and staining character up to the time of segmenting of the spireme. When lightly stained one or more vacuoles or lighter staining areas are often visible in it (figs. 59, 63). Between the time of formation of the chromosomes and the completion of the spindle the nucleolus, as a unit, disappears though fragments with the same staining qualities are still seen within or without the nuclear area (figs. 65, 66, 67). It may be recalled here that similar dark stained granules are visible in the cytoplasm while the nuclear wall is still intact (figs. 60, 62, 64). Hence it is not certain that any of the dark bodies seen in the cytoplasm during mitosis are of nucleolar origin. During the progress of synapsis a peculiar concave sheet or disk is found lying against the inner surface of the nuclear wall, often on the side opposite the synaptic knot (figs. 59, 60, 61, 62). This disk is .5 μ thick and often 10 μ across. This body is first seen when the nucleus is just entering synapsis (fig. 60) and was not seen after the segmenting of the spireme (fig. 63). Structures which may be remains of these disks were occasionally found lying near the poles of the spindle, but the identity of these is not certain (fig. 65). This disk stains a deep black with iron haematoxylin and a dark gray with Flemming's triple stain, and in structure it may appear in section as a clear black line or, in other cases, it is rough in outline (figs. 59, 60, 62). The morphological identity of this disk has not yet been determined. Further work on the origin and fate of this disk is planned as part of a detailed study of the whole process of reduction in the embryo sac mother-cell.

A marked segregation, of the at first nearly homogeneous cytoplasm of the mother-cell, forming 2 concentric layers around the nucleus, usually occurs during the progress of synapsis. The outer layer, of 4 or 5 μ in thickness, is highly vacuolated, stains weakly and apparently plays only a minor part in connection with the divisions of the nucleus and cell (figs. 60, 62, 63, 64). The inner layer of cytoplasm is but slightly vacuolated, is densely granular, stains darkly and becomes highly active at the time of nuclear division. This is indicated by the numerous strands and fibers formed in it (figs. 63, 65, 67, 68, 70). With the growth of the embryo sac, after the disappearance of the cell walls between the first four nuclei, this differentiation of the cytoplasm becomes gradually less marked (figs. 73, 74) and with the increased vacuolation of the eight-nucleate sac this differentiation of the cytoplasm becomes scarcely discernible (figs. 76, 77). When, however, in the sixteen-nucleate sac, the egg and synergid nuclei are cut off, and the endosperm-forming nuclei are grouped closely together, the cytoplasm about the egg and synergids and about the group of endosperm nuclei again becomes denser (figs. 84, 88). At no later time, however, does it show evidence of the extreme activity seen during the two reduction divisions. When the mitotic figure is organized in the embryo sac mother-cell, after the occurence of the process of synapsis and chromosome-formation described above, the axis of the spindle may be either longitudinal or transverse to the ovule (figs. 65, 66). This spindle is bipolar at the earliest stage seen, acute at the poles and about 15 μ long by 10 μ across. The chromosomes are more or less bent rods, only about twice as long as thick, and are about 12 or 14 in number. In the later anaphase the chromosomes are seen to be somewhat angular but nearly isodimensional. They then measure 1 to 1.5 μ in diameter and each has attached to the side turned toward the pole a bundle of smaller fibrils (fig. 65).

The daughter nuclei from the first division are apparently organized very promptly after the chromosomes reach the poles. These nuclei are 7 or 8 μ in diameter, *i. e.*, about half as large as the parent nucleus in late synapsis. The chromatin of the daughter nucleus, at the time the new wall is first developed, has the form of a series of more than 12 rather angular granules. These deeply staining granules lie near the nuclear wall and are more or less connected by faintly colored strands (fig. 67). Before the nuclear wall breaks down, in preparation for the second division, the chromatin assumes a more uniformly thread-like arrangement and, though larger granules are still evident the meshes become more numerous and regular (fig. 68). A cell plate is often, perhaps always, formed on the spindle between these two nuclei (fig. 67).

The tetrad stage was the next stage of the development of the embryo sac of which an adequate number of examples were seen for determining the further history of these nuclei. In the young tetrads two spindles are seen usually at right angles to each other, and a well-marked cell plate is present on each (fig. 69). The nucleus at this time is 9 or 10 μ in diameter; the chromatin net is rather open and of slender threads with many small granules. The nucleolus is again evident now, for the first time since late synapsis, as a rounded, darkly staining body, about 3 μ in diameter (fig. 69). In many slightly older sacs the cell plates have been replaced by complete, though delicate, cell walls. The cytoplasm is thus cut to a tetrahedrally arranged group of cells, with (evidently), haploid nuclei (figs. 70, 71, which show adjoining sections of the same tetrad). This group has the characteristic structure, and, in the many features noted, the complete cytological history of a spore tetrad. It seems therefore impossible to escape the conclusion that this is a *tetrad of megaspores*, directly comparable with the tetrad of microspores formed in the anther of this *Peperomia* and with the linear row of four megaspores formed in many angiosperms. (See Johnson, 1907, p. 1; Coulter, 1908, pp. 363-4; Brown, 1908, p. 453). In only a few of the many cases observed were the four nuclei and the resulting cells arranged more nearly in a row (fig. 72). The question arises here whether this tetrad of megaspores is a primitive, fern-like feature, retained by this *Peperomia*. If so from what ancestor may it have been derived? On the other hand, if it is secondary in origin to what influence may this return from the serially arranged megaspores characteristic of nearly all seed plants to the tetrad arrangement found in pteridophytes be attributed? The consideration of these questions may be taken up later on, in our general discussion of results.

The walls separating the young megaspores are very delicate, so delicate in fact that one sees at first only a cleavage plane, made more evident by a slight shrinkage (fig. 70, 71). The wall itself can, however, usually be discovered in these cases, as a dark line next to one cytoplast or the other. At a slightly later stage of development these delicate tetrad, or spore walls have disappeared and the cytoplasm once more becomes a continuous mass throughout the embryo sac (figs. 73, 74). That is the protoplasts of all four megaspores fuse to form one composite mass of continuous cytoplasm, enclosing the four megaspore nuclei (see Lloyd, 1902; Brown, 1908, p. 449; Fisher, 1914, p. 143). It is quite possible that walls are not formed in all tetrads, though it is surely the rule. In figure 69, *e. g.*, is shown a sac of large size in which the central vacuole has begun to form and in which the cell plates seem to be disappearing without giving rise to walls. From this first divergent step onward the development of this embryo sac differs greatly from that of other angiosperms, the chief peculiarity being that all the descendants of all 4 megaspore nuclei participate in forming the mature embryo sac.

The next step toward the development of the ripe embryo sac is the formation of a mitotic spindle by each of the four free nuclei. These appear very soon after the disappearance of the megaspore walls and before any other change occurs in the sac except a slight growth in length, a separation of the 4 nuclei and the formation of a central vacuole (figs. 73, 74). The spindles are located in the peripheral portion of the cytoplasm and the axis usually lies parallel to the neighboring portion of the wall of the sac (figs. 75, 76). The axis of the spindle may have any position in relation to the axis of the sac itself. The eight nuclei resulting from this division were connected, in many of the cases seen, by the persistent fibers of the four spindles. No cell plates could be found, however, nor could evidence be obtained of the separation of these 8 nuclei by even temporary cell walls (figs. 76, 77). This absence of plates, as has been pointed out by Brown (1908, p. 445), indicates that this is the equivalent of the free nuclear division found at the germination of the megaspore of most angiosperms. Smith (1911, p. 216) finds however that a cell plate is formed at the first division of the embryo sac of *Clintonia*. The eight resting nuclei at this stage are each 8 to 10 μ in diameter. Each has a nucleolus 2 or 3 μ in diameter and a wide-meshed, peripheral chromatin net not unlike that of the spore nuclei (fig. 77). During the division of the megaspore nuclei the sac increases in size and at the 8-nucleate stage has a width of 40 or 45 μ and a length of 60 μ or over. The micropylar end of the sac is narrowed to a pocket of less than half the diameter of the opposite end. In this micropylar pocket lie two nuclei derived from the nucleus of the micropylar spore of the tetrad, while just below the middle lie the three pairs of nuclei derived from the other three spores (fig. 77). The embryo sac at this stage has become more distinctly bipolar than in the four-nucleate stage, and it continues from this time on to the ripe seed to show this definite polarity.

The early phases of the preparation of these 8 nuclei for the next division have not been seen. The late telophases of this mitosis, which were seen, show 16 reorganized nuclei, connected in pairs by strands, which are evidently spindle fibers, closely like those shown in the 8-nucleate stage (figs. 76, 77). When these 16 nuclei are first formed, 4 of them lie in a pretty compact group in the micropylar pocket of the sac. The other 12 nuclei lie near or below the middle, often in 3 distinct groups of 4 each (fig. 81). It is clear from the arrangement of these nuclei that the 4 at the micropylar end have come from the micropylar spore and each of the other quartets from one of the other three spores. In a series of thin sections it is not always evident that the 16 nuclei are grouped in tetrads, but careful study of the series usually shows them to be so. At a slightly later stage of development two of the micropylar nuclei are found surrounded by denser protoplasm and cell walls, thus forming two cells that fill this end of the sac. The larger of these, the egg, has about twice the bulk of the second, which is the single synergid. In some cases these two cells may lie side by side (figs. 82, 89, 104) while in other cases the synergid may lie somewhat above the egg (figs. 84, 88, 98, 102). The egg in the mature embryo sac is often 25 μ long, and has a series of vacuoles outside the denser zone of cytoplasm immediately surrounding the nucleus. The nucleus of the mature egg may be 12 μ in diameter and has a distinct, rather fine chromatin reticulum and one nucleolus (fig. 81). The synergid is somewhat similar in organization but is usually smaller and has a less dense cytoplasm (figs. 81, 82, 84, 88). The remaining 2 of the 4 micropylar nuclei usually move downward, at about the time the cell walls appear around the egg and synergid. Later these two free nuclei are found close to the other 12 nuclei that lie in the lower half of the sac. In a few of the cases all four of the micropylar nuclei were still lying in the pocket after fertilization had occurred. Cell walls could not be seen about them and their exact origin and relation to each other could not be made out (fig. 87). It is of course conceivable that one or more of these nuclei may have come in from the pollen tube.

These 14 nuclei of the lower end of the sac at first lie in one or two groups in the peripheral sheet of cytoplasm that surrounds the single, large, central vacuole (figs. 80, 83). Later the cytoplasm becomes more abundant and the vacuoles smaller, more numerous and more peripheral in position. The nuclei are then found imbedded in a larger, denser mass of cytoplasm that usually lies near the center of the embryo sac (figs. 82, 84). Thus far there is no indication, with one or two possible exceptions to be mentioned later, of the separation of any of these 14 nuclei from their fellows by cell walls. Not even cell plates have been found. But it must be noted that no cell plates were actually seen in the cases of the divisions forming the egg and synergid, whose walls probably arise from a cell plate, as Brown (1908) has found them to do in the case of *Peperomia sintenisii.* It is therefore possible that embryo sacs of just the proper stage would show more or less definite cell plates on the spindles of the sac in this last division. Such walls, if present, would correspond to the cell plates that form the walls of the peripheral cells of *Peperomia pellucida* (Johnson, 1900*a*) and of *P. sintenisii* (Brown, 1908). Such peripheral cells are rarely formed in our species, only 3 or 4 instances being noted (figs. 85, 105).

3. *Fertilization and formation of the endosperm nucleus.*

At the stage just described with two micropylar cells and the more central group of 14 nuclei, the embryo sac is mature and ready for fertilization (figs. 78, 80). The 14 nuclei may possibly not become closely grouped until after the male nucleus has entered the egg. At least, since the earlier phases of fertilization were not observed, it can only be said that all cases where the male nucleus was present in the egg the endosperm nuclei of the sac were closely grouped. The finding of structures in the style which can be identified with nothing but pollen tubes makes it practically certain that fertilization is accomplished in the ordinary way by the entrance of one male nucleus into the egg. A few cases were noted in which two nuclei were present in the egg after the penetration of the pollen tube into the micropyle. Many other eggs were seen with a nucleus containing two nucleoli (figs. 82, 83, 84, 98, 104) or a single nucleolus of double size (figs. 88, 89, 102). The nucleolus is always single except in these presumably fertilized eggs and in the primary endosperm nucleus or its derivatives. All these facts indicate that fertilization is normal in this *Peperomia.* No evidence was discovered that the second male nucleus enters into the endosperm-forming complex. This phenomenon has been sought for in each species studied but without discovering it in any of them.

As the nuclei become compacted into the group that form the endosperm nucleus the cytoplasm immediately about them grows denser. In the cytoplasm throughout the whole lower portion of the sac there appear at this time numerous droplets of oil, varying from 1 to 6 or 7 μ in diameter. These bodies are deep black, in material fixed in Flemming's fixing fluid, and then sectioned and examined without decolorizing. They take the same color when sections of seeds preserved in formalin are treated with dilute osmic acid. This staining quality together with their behavior toward alkanna and their structure, leave little doubt that they are oil globules that probably serve as a temporary reserve food material.

Unless fertilization occurs in an egg, which may be indicated by the number or size of its nuclei and nucleoli, development throughout the whole embryo sac, and indeed in the whole seed and fruit, ceases completely. Considerable numbers of ovules in which the sacs have ceased development and have begun to degenerate, are seen on spikes where other ovules are developing normally. More rarely all, or nearly all of the ovules initiated degenerate before passing far beyond the 16-nucleate stage.

The next evident step in the further development of the embryo sac after the appearance of two nuclei in the egg is the formation of the huge endosperm nucleus. The fusion of the 14 nuclei which give rise to it is usually preceded by a crowding together of these components, until many of them are flattened against each other on one or more sides (figs. 84, 86, 87). Occasionally fusion of the nuclei in pairs may begin while the different pairs are still far apart about the vacuole (fig. 83). As the nuclei of the central group, formed in the more usual type of fusion, become compacted those in the center of the group begin to lose identity by the disappearance of their walls where in contact. There are thus formed oval or lobed nuclei, with two or more nucleoli (figs. 83, 85, 86, 87). The number of lobes, and especially the number of nucleoli, indicate clearly the number of nuclei that have fused. To the first fusion products, made up of 2 or 3 nuclei each, other single nuclei may be added or two of the fusion products may themselves melt together. Thus gradually, all of the 14 nuclei lose their individuality in the at first lobed, and often vertically flattened, primary endosperm nucleus (figs. 87, 88, 89).

Usually, at the time the nuclei come into contact, the chromatin has the form of a reticulum, though at one stage there seems to be a rather simply folded or looped chromatin thread or skein (figs. 84, 86). At the surfaces where the nuclei are in contact there is soon evident a distinct massing of the chromatin threads. This is first seen over the area of contact, and then, as the walls between the two nuclei disappear, the chromatin becomes especially abundant in a ring about the opening through the perforated wall (figs. 87, 93, 94). Thus the chromatin reticulum does not appear to degenerate over the surface of contact, but opens out and comes to lie chiefly on the surface of the new nucleus. Part of the chromatin may, for a while after fusion, be found in the interior of the fusion nucleus. Finally the composite chromatin net comes to be pretty evenly distributed over the surface of the large endosperm nucleus. This reticulum may at first show meshes of very different sizes in different parts. These correspond in size to the different sizes of mesh seen in the different contributing nuclei before fusion. They indicate clearly that the choromatin contributed by each fusing nucleus maintains its identity for some time at least, in the primary endosperm nucleus so produced (fig. 84). Whether the smaller of these fusing nuclei play an equal part with others in the later activity of the fusion product was not determined. The fusion of nucleoli does not occur as quickly, is not carried as far as that of the nuclei, and there are always several, often 6 or 8 nucleoli in the endosperm nucleus. The number of these nucleoli present and the size of some of them indicate that the larger ones are made up of 2, and sometimes of 3 or 4 fused nucleoli (figs. 84, 86, 88). Before the fusion of the nuclei is completed, however, the number of the nucleoli begins to increase, until sometimes nearly 30 are present. These new nucleoli are apparently formed by simple budding of the large fusion nucleoli (figs. 92, 95).

4. *The endosperm and embryo.*

The egg, and likewise the whole contents of the embryo sac and seed, degenerate, as noted above, unless fertilization is accomplished. The occurrence of fertilization is indicated by the presence of two nuclei, of two nucleoli, or of a double-sized nucleolus in the egg and by the formation of the composite endosperm nucleus. The next evidence of further development in the sac, aside from the continued growth of all its parts, is the division of the endosperm nucleus. The first division of the egg itself does not usually occur until 8 or 10 endosperm cells have been formed.

The primary endosperm nucleus, when the fusion of its 14 constituent nuclei is finally completed, is a rounded, though often somewhat flattened and lobed structure. It is very large, having a diameter of 20 to 25 μ. It has a rather coarse and darkly staining, superficial chromatin reticulum, and, before its first division may have as many as 25 or 30 nucleoli. The latter differ greatly in number, size and shape which differences are evidently due to the fragmentation noted above of the 14 nucleoli contributed by the fusing parent nuclei (figs. 92, 95). The resting period of this nucleus is apparently not a very long one. Relatively few examples were found where the constituent nuclei were completely fused and the product so formed was still undivided. There were many scores of examples of fusing nuclei to a few dozens of those undivided or in division.

The first spindle organized in this fusion nucleus is a very large one, with rather broad ends and very numerous chromosomes, which are crowded together at the equator to form a plate often 15 or 20 μ in diameter (figs. 98, 99). The axis of this spindle is usually approximately transverse to the ovule and the dividing wall which is at once formed on its equatorial plate is nearly longitudinal to the ovule (figs. 98, 99, 102, 105). This wall, however, may make any angle with the sagittal plane of the ovule itself. The number of chromosomes formed on this spindle and on the later ones of the endosperm is far greater than in any other mitoses of this species. They are, however, so crowded that it was impossible to count them with certainty. In all cases where the number could be made out approximately it was found to be far above a hundred (figs. 98, 99, 106). This makes it altogether probable that the 144 or 196 chromosomes of the 14 contributing nuclei retain their individuality in the resulting endosperm nucleus. The form of the chromosomes is at first rod-like, rather elongated and sometimes bent. Later they become shortened and rounded (figs. 98, 99). The two new nuclei, which are promptly organized about the poles of the spindle, are but slightly smaller than the endosperm nucleus. They are usually decidedly flattened in the plane of the new cell wall which is immediately formed between them. They are also often more or less lobed in the manner of the primary endosperm nucleus (figs. 102, 103). In fact the wall of the daughter nuclei is often so very irregular at first that in sections it looks as if torn or incomplete (figs. 102, 105). The chromatin net of these nuclei resembles that of the primary endosperm nucleus, except that it is more nearly homogeneous, in size of threads and meshes. During the first mitosis the nucleoli disappear (figs. 98, 99), but they soon reappear in the daughter nuclei, in numbers ranging up to a dozen or more each (figs. 102, 106). As in the later phases of the primary nucleus so here the nucleoli differ greatly in size, some having ten times the diameter of others (figs. 103, 104, 105).

The spindles for the second mitosis in the endosperm are usually nearly longitudinal, and the walls formed are somewhat transverse to the ovule (fig. 104). The formation of the cell wall between the nuclei, in this as in the first and in all later divisions in the endosperm, follows immediately on nuclear division. The four endosperm cells resulting from the second division usually divide next by spindles perpendicular to those of that division, and hence by walls longitudinal to the ovule. Later a number of more or less transverse walls appear and there is thus formed an endosperm which, in the ripe seed, consists of about 40 or more cells arranged somewhat regularly in 3 or 4 tiers (fig. 108). The numbers just given are for the endosperm and embryo shown in figure 108, which is the largest found among scores of the ripest attached or fallen seeds collected by the writer. In figure 110 are shown the endosperm and embryo from a seed that was 10 or 12 days in damp moss after being collected, before being dropped into formalin and then sectioned. The embryo is farther developed than the one in figure 108, and the endosperm shows 27 cells in the single section. It seems probable that the difference in stage of development of these two seeds should be counted as due to the beginning of germination in the latter, though it is possible that development may sometimes go this far before the fruit falls from the plant. In the ripe seed these endosperm cells have irregular nuclei of 10 to 20 μ in diameter, with numerous nucleoli, and have rather densely staining protoplasts. Scattered through the cytoplasm of the endosperm, from the one-celled stage onward, are found quite numerous rounded bodies of the size of the nucleoli, which, like those of the mature embryo-sac, are colored black by Flemming's fixing fluid. This fact, together with their appearance, and their continuity from the mature sac onward, indicates that they are of the same sort of oil or fat that was demonstrated in the sac. These globules seem to diminish in size as the endosperm cells multiply. In the mature endosperm of this species, as in *P. pellucida*, stored starch, so far as the iodine test shows, is entirely wanting.

The embryo.—From the time the 14 contributing nuclei are grouped for the formation of the primary endosperm nucleus until their fusion is completed, two nucleoli are found in the single nucleus of the egg. No cases were found where the distinct male and female nuclei could be seen in the oospore, as they were in *P. pellucida.* The two nucleoli of the oospore remain distinct and separate until the first division of the endosperm nucleus has been completed, at about which time they apparently fuse to one. The nucleus and nucleolus of the oospore both remain single from this time onward, usually until 10 or 12 endosperm cells have been formed (figs. 84, 104).

The oospore continues to grow and, at some time after the number of endosperm cells has reached 8 or 10, it begins to divide by successive walls whose sequence has not been made out. There is thus formed a small embryo which in the ripe seed shows but 8 or 10 cells in a longitudinal section (fig. 108). In the seed referred to above, which had passed two weeks in damp moss after being gathered, the form of the embryo is some what more elongated and the cells are more numerous. This probably represents the more usual form of the embryo, for this figure was drawn from sections of formalin-fixed material, which gave no evidence of shrinkage such as was seen in nearly all older embryos that had been through alcohol (fig. 110). The shaded cell at the right of the embryo in the latter figure is probably the persistent synergid. The condition of embryo and synergid in the ripe seed is thus seen to be similar to that found in the seed of *Peperomia pellucida* (See Johnson, 1900, figs. 12, 13, 14).

F. GERMINATION OF THE SEED

After searching repeatedly for germinated seeds in the field, and attempting to germinate collected and partially dried seeds in the laboratory, success was at last attained with seeds that were placed in damp moss at once on gathering, and mailed from Jamaica to Baltimore. These reached Baltimore on February 11, 1914, and were at once placed between layers of damp filter paper in a closely covered glass jar. On March 25 the radicle of the embryo was seen projecting from several of the fruits (cf. Johnson, 1902, fig. 36). In the three weeks following some of the seeds had developed a hypocotyl twice as long as the seed and a primary root three times as long (fig. 112). Examples of all stages were fixed and preserved for surface study and sectioning.

The first external indication that germination is going on is the pushing out of the wall of the fruit at the upper end. Soon the white, pearly layer of the endosperm becomes visible between the 4 or 5 parted flaps of the carpellary tissue (fig. 112). At first the endosperm forms a complete jacket about the embryo and is commonly capped by the pushed-off stylar remnant (fig. 111). Somewhat later the tip of the radicle bursts through the micropylar end of the endosperm and continues to grow outward till the root cap, the primary root and at length the hypocotyl are differentiated and exposed (fig. 112). During all this time the endosperm remains tightly clasping about the cylindrical primary axis of the plantlet like a collar (fig. 112, and Johnson, 1902, fig. 36). Ultimately the cotyledons slip out of the clasping endosperm and spread apart to allow the expansion of the rudiments of the plumule at the stem growing point, just as in *P. pellucida* (Johnson, 1902, figs. 38, 39). The outer surface of the embryo itself shows at the very tip of the root, at a time shortly before the cotyledons have escaped, a delicate root cap about two diameters long. Behind this there are scores of root hairs of lengths reaching up to a millimeter or more. Then, at the upper limit of root hairs, there is an abrupt swelling of the axis to form the hypocotyl, which has nearly twice the diameter of the root. The surface of the usually sharply bent hypocotyl is sprinkled with hydathodes and with still more abundant oil-containing cells but no stomata are visible at this early stage (fig. 112). The cotyledons at the time of their escape have an epidermis of rather wavy margined cells with stomata, hydathodes and oil cells scattered among them.

The study of sections of the embryo during germination shows that the embryo is differentiated in the usual way from the rounded cell mass present in the ripe seed. This globular body elongates, and broadens at the chalazal end. Then two lateral prolongations of this same end give rise to the cotyledons and leave between them a depressed area from which the stem growing point is soon formed (fig. 111. See also Johnson, 1902, figs. 34, 35). The vascular system consisting of a single axial bundle from the tip of the root to the base of the cotyledons, is developed in the usual way, so far as followed. The mode of transition was not followed out because of the lack of satisfactory series of sections of this region. The behavior of the endosperm during germination is very similar to that already pointed out by the writer in Saururus and in other Piperaceae (Johnson, 1900*b*, figs. 7–10, and 1902, figs. 30–39). The growth of the endosperm is more active at the sides and it elongates most in the direction of the axis of the seed (fig. 111). The micropylar end of the endosperm closes together partially, at first, about the tip of the radicle. The chalazal end, which in the ripe seed consists of 3 or 4 layers of cells below the embryo, very soon thins out to leave but 1 or at most 2 layers to separate the tips of the two cotyledons from their food supply in the perisperm (fig. 111). Whether this latter thinning out is due entirely to the crushing and absorption of the cells of this part of the endosperm by the embryo, or may be due in part also to the displacement of these cells was not determined with certainty. It seems to be due chiefly to the former process. This thin layer of tissue, between the absorbing tips of the cotyledons of the embryo and its food supply in the perisperm, has an evident advantage in allowing the more ready transfer of this material to the embryo.

The character of the contents of the chalazal endosperm cells remains similar to that of the endosperm cells of the mature seed. The protoplasm is dense with small vacuoles and the nuclei are large, characteristically irregular in shape, and may have 8 or 10 nucleoli each. It seems clear from their persistence in this position, and from the characters just mentioned that these cells must serve to pass on nutritive material from the perisperm and it is highly probable that they serve the further function of actively absorbing and digesting the contents of the perisperm cells. In other words the scantily developed endosperm, though containing little stored food itself, continues to serve as nurse for the embryo till the supply of food in the perisperm has been handed over to the young sporophyte and the rooted seedling becomes self-dependent (see Johnson, 1902, p. 334). The middle portion of the barrel-shaped portion of the endosperm remains thickest and serves as a plug to stop tightly the opening in the disrupted end of the fruit, and so probably completely prevents the entrance of water or fungi from without and likewise the escape of any dissolved food material present between the endosperm and perisperm. The cells of the upper exposed half of the endosperm mass, especially the outer ones, are much larger than those below. They also have much larger vacuoles and where exposed have thick outer walls. These characters all seem to make this part of the endosperm jacket a more adequate protection against desiccation or other injury to the delicate embryo within (fig. 111).

When the endosperm has reached a length of 600 or 700 μ it ceases to grow, and as the embryo continues its elongation the radicle is forced through the opening in the endosperm at the micropylar end, pushing aside such remains of the parietal tissue and style as may have been clinging to this end of the endosperm jacket.

G. ABNORMAL EMBRYO SACS

In addition to the exceptional occurrence of peripheral cells and of a second synergid, which were referred to in discussing the normal development of the embryo sac, because they show a resemblance, of *P. hispidula* to other species, there is a less common, probably decidedly rare, aberration still to be mentioned. This has not been described or figured in discussing the normal development for the sake of avoiding confusion. This peculiarity is the occurrence of archesporial cells, megaspore tetrads and embryo sacs in pairs or trios in the same nucellus. The occurrence of more than one archesporial cell in a nucellus has been noted in a large number of species of angiosperms since they were discovered by Strasburger (1879) in *Rosa*, and by Fischer (1880) in a half dozen other genera of the Rosaceae. Not only is this true, but in the cases of *Fagus*, *Corylus* and *Carpinus* (Benson, 1894), of *Delphinium* and *Senecio* (Mottier, 1893, 1895), and some others, two or more embryo sacs may even mature until ready for fertilization.

The sort of doubling of the megaspore and embryo sac just referred to occurs somewhat rarely in *P. hispidula*. In the youngest stages seen parietal cells were already formed. The most frequent type of doubling found is that where the twin megaspore mother cells or embryo sacs are side by side and each has essentially the structure of the ordinary single cell or sac (fig. 113). In other cases, however, two archesporial cells may lie one above the other in the axis of the ovule (fig. 114), and, still more rarely three of these cells may occur in one longitudinal row (fig. 115). The appearance of these groups of cells, especially the pairs or trios in longitudinal rows, suggests of course the possibility of there being rows of megaspores formed by the division of a mother-cell. But this possibility is practically excluded by the fact that the stage shown in figure 113 is too early to show such a division, while in the cases given in figures 114 and 117 the nucleus of one cell in each case shows clearly the typical synapsis characteristic of the maturing megaspore mother-cell. There can be no doubt that the cases mentioned really have twin megaspore mother cells. It is probable also that the cases shown in figures 115 and 116 are really groups of megaspore mother cells rather than of megaspores themselves.

The further fate of these multiple archesporial cells is also proof of their real nature. A considerable number of examples were seen of the further development of the paired archesporial cells of both types. Thus figure 118 shows two superposed embryo sacs which have evidently come from an archesporial pair like that shown in figure 114, one of which has given rise to a sac with four megaspore nuclei, while the other has six nuclei in it. Sometimes the disparity in rate of

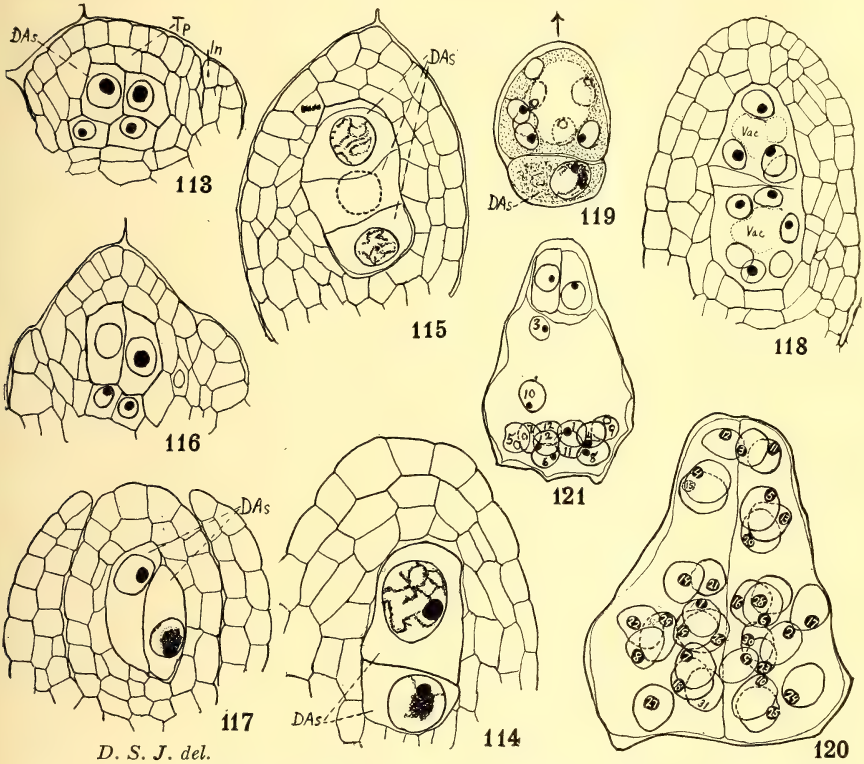

FIG. 113. Longitudinal section of a young ovule containing 2 or possibly more, embryo sac mother-cells in a transverse row. ×400.

FIG. 114. Similar section showing 2 mother-cells in the axis of the ovule, the lower one in synapsis. ×600.

FIG. 115. Similar section with 3 mother-cells on the same axis. ×400.

FIGS. 116, 117. Similar sections of the type shown in figure 113. ×400.

FIG. 118. Similar section of the type of abnormal sac shown in figure 114. The upper sac has but 4 nuclei while the lower has 6. ×400.

FIG. 119. A slightly oblique longitudinal section of a later phase of the same type. One sac has 8 nuclei, the other is uninucleate. ×400.

FIG. 120. Longitudinal section of nearly mature embryo sacs, one with 15, the other with 16 nuclei. These are numbered in the order of appearance of their nuclei in focus. ×400.

FIG. 121. Similar section of a mature embryo sac, containing but 14 nuclei altogether. ×400.

development is still greater as in the case caught by the somewhat oblique section shown in figure 119. All of the mature or nearly mature twin sacs seen were of the collateral type shown in figure 120, in which each of the embryo sacs, except for the presence of but 15 nuclei in the left one, has exactly the structure of the normal single sac at this stage. The lack of one nucleus out of the 16 in the last sac referred to exemplifies another type of abnormality not uncommon in the otherwise normal single sacs. It is possible of course that the failure to find 16 nuclei in certain evidently mature sacs may be due to the difficulty of counting the crowded endosperm nuclei. This difficulty is increased by the fact that the nucleoli, which must be largely depended on in counting, may occasionally be displaced from the surface of the section and lost. Embryo sacs are not uncommonly found in which but 13, 14 or 15 nuclei can be counted even when the egg and synergid are differentiated. Figure 121 shows such a sac in which but 14 nuclei could be discovered, including those of the egg and synergid.

The ultimate fate of the paired embryo sacs mentioned has not yet been determined. From the occurrence of a number of cases like that shown in figure 120 it seems probable that two eggs may be fertilized in one ovule and thus two embryos be formed. No case of this sort has been seen in *P. hispidula*, but seeds of *P. pellucida* have been seen from which two embryos protruded on germination. It may be discovered that these are developed from separate twin embryo sacs in the same ovule, such as have been described for *P. hispidula* but have not yet been reported for *P. pellucida*.

The occurrence of these abnormal embryo sacs gives us no indication of the phylogenetic origin of the peculiar composite sac normally found in this and other species of *Peperomia*. Neither of the two methods of doubling here found can be conceived of as in any way explaining the origin of the simple 16-nucleate sac from the 8-nucleate one characteristic of most angiosperms. This is evident from the fact that each of the twin mother cells, by itself, gives rise to a complete 16-nucleate embryo, and from the fact that in both the normal case and in the case of the twins it is one complete megaspore tetrad that gives rise to each complete individual sac.

The widespread distribution among the angiosperms of the genera in which these multiple archesporial cells and embryo sacs occur is practically conclusive evidence that they can have no value as indicators of phylogeny aside from supporting the well-established view that all angiosperms are derived from archegoniates with a many-celled archesporium in the megasporangium.

Finally it might be noted that though the causes producing these abnormal double sacs were not discovered it was found that the causes seem evidently confined to relatively few plants. Each of the abnormal plants may bear several of the double embryo sacs in the same spike, along with normal ones. The mature embryo sacs with two or three nuclei less than normal probably owe their peculiarity to some minor disturbance of nutrition which inhibits nuclear division before all of the megaspore nuclei of each sac have completed the second division.

THEORETICAL CONCLUSIONS

The peculiarities in the structure and development of the vegeta- and reproductive organs of *P. hispidula* recorded above lead to certain conclusions concerning the meaning and phylogenetic origin of these peculiarities. The most important secondary question which these facts may help to solve is that of the comparative primitiveness of this plant in relation to other members of its genus and family and to other angiosperms.

We will consider the bearing of the facts here recorded under the following heads: (1) Vegetative structure. (2) Structure and development of the stamen flower and fruit. (3) Development of the archesporium and megaspores (cell plates etc.). (4) Development of the embryo sac including division of megaspore nuclei, formation of the egg apparatus, endosperm nucleus and peripheral cells. (5) Development of embryo and endosperm. (6) Germination and food-storage in the perisperm. (7) Relative primitiveness of the 8-nucleate and 16-nucleate embryo sacs.

1. *Vegetative structure.*—As was stated above, the structure of the vegetative organs of *P. hispidula* approaches in many features the simplest types known for these organs among dicotyledons. This is shown not only in the habit or organization of the plant as a whole but by the intimate structure of each of the individual organs. Thus the root is small, sparsely branched and has a single very simple vascular bundle. The stem likewise is relatively short and little-branched, is delicately herbaceous and has a simple internal structure, although the single, closed, vascular bundle is slightly more complex than that of the root. The leaf though furnished with trichomes, hydathodes and oil-cells on the surface has, aside from its three water glands, the simp-

lest sort of internal structure with slightly specialized palisade cells and but one, or in places two other layers of mesophyll cells. All in all the internal structure of this *Peperomia* is the simplest thus far described within the genus. This means also that it is the simplest within the family.

The question arises as to whether this simplicity is primitive or not. The more generally accepted views of the character of primitive angiosperms, which were probably dicotyledonous, conceive them to be tree-like or shrub-like woody plants (Wettstein, 1911, p. 196; Arber and Parkin, 1907; Maneval, 1914; Engler and Gilg, 1912; p. 156). It is clear that all these writers must regard this *Peperomia* as far removed in vegetative structure from the most primitive type for the dicotyledons. The tree-like *Pipers* though not the most primitive dicots in stem structure seem clearly the more primitive members of the Piperaceae. *P. hispidula* on the other hand must be regarded as among the most highly specialized members of this family, in most points of vegetative structure.

2. *Development and structure of flower and fruit.*—In treating these topics it will not be necessary to discuss in detail the much disputed question of the relative primitiveness of unisexual and hermaphrodite flowers among angiosperms nor to decide whether naked flowers are sometimes primitive, as well as sometimes due to reduction. One or two points showing the relative simplicity of this *Peperomia* in its own family seem reasonably clear. In the first place all the flowers are potentially hermaphrodite and there is no variation in the number of microsporangia formed in each stamen, such as occurs in some other Piperaceae (Johnson, 1910). This number, however, is but two, a very unusual number among angiosperms. The same number of microsporangia per stamen is found throughout the genus *Peperomia*, while in other Piperaceae, and in nearly all other angiosperms, four sporangia are found in each stamen. Evidently *P. hispidula*, along with the others of its genus, is decidedly aberrant or specialized in this respect. The development of the stamen, of the microspore, and of the male prothallus is like that of the vast majority of angiosperms.

The history of development indicates very definitely that the carpel of *P. hispidula* is single, as there is not the slightest trace of its having a compound origin. Yet the carpels of its nearest allies the *Pipers* show rather definite evidence, in the presence of three lobes in the stigma and of six vascular bundles in the wall of the ovary, that the latter is composed of three carpels (Johnson, 1902, 1910). *Saururus* also (Johnson, 1900*b*), which is still regarded by Engler as the most primitive of the Piperales, shows a similar compound ovary. In other words, the carpel also seems simplified through specialization by reduction from the more primitive type characteristic of the Piperaceae in general. The mature carpel or fruit is thin and of few layers of tissue as compared with the *Pipers* studied. Not the slightest evidence has been found that any floral envelopes were ever present in this plant. The above-mentioned evidence points to this and other *Peperomias* as being forms with flowers more specialized than those of the related *Pipers*.

In the single ovule of *Peperomia*, we have, as has been pointed out by Fisher (1914, p. 148), another character which cannot be regarded as primitive by those accepting any of the current views of the relationship of the Piperaceae (see Engler and Prantl, p. 189; Hallier, 1905; Lotsy, 1911, pp. 487 ff.; Engler and Gilg, 1912, pp. 157, 158). It must of course be kept in mind that while the number of ovules per carpel is rather constant in some families there are others like the Rosaceae where the number may vary from one to several.

3. *The development of archesporium, megaspores and cell plate.*—The hypodermal primary archesporial cell is usually single and by its division gives rise to a definitive archesporial cell that becomes the mother-cell of the embryo sac, and above this a tapetal cell that forms several layers of tapetal tissue in the mature seed. The definitive archesporial cell and its nucleus enlarge to double their original size. The nucleus after showing a typical synapsis undergoes 2 mitoses by spindles perpendicular to each other giving rise thus to four, probably haploid, nuclei arranged at the points of a tetrahedron within the nearly spherical embryo sac. Delicate cell walls then arise from cell plates of the usual type, forming thus 4 tetrahedral megaspores, comparable in appearance and origin with the microspore tetrad of this species, or, except for the delicate character of the walls, with the megaspore tetrad of *Selaginella*. The whole development of these 4 cells, including the chromosome history of the nuclei as far as followed, shows them to be the exact equivalents morphologically of the megaspores, which in most angiosperms are arranged in an axial row (see Coulter, 1908, p. 364). The unusual arrangement of megaspores in a tetrad in this *Peperomia* is probably to be associated with the somewhat rounded form of the spore mother cell, and this depends,

perhaps, on the form of the ovule (see Johnson, 1900, p. 2; Brown, 1908, p. 453; Stephens, 1909*b*, p. 383; Brown and Sharp, 1911, p. 446; Fisher, 1914, pp. 421–430). That the form of the ovule and embryo sac and the arrangement of the contents of the latter may be related to each other and may vary considerably in the same plant is shown by this *Peperomia* itself. Thus when the 4-nucleate embryo sac is about isodimensional the nuclei are arranged at the corners of the tetrahedron, while in other cases where the embryo sac is twice as long as broad the nuclei are in 2 subterminal pairs (fig. 72.) Of the external and internal causes determining the form of the ovule and the associated form and arrangement of the embryo sac we know almost nothing (Brown and Sharp, 1911, p. 446).

As for the phylogenetic origin of this tetrad arrangement of the megaspores of *P. hispidula* it seems clear that it is a peculiarity developed within the genus, for the allied and probably more primitive genera *Piper*, *Heckeria*, *Saururus*, etc., have the megaspores or megaspore nuclei arranged in a row (see Johnson, 1900*b*, 1910; Fisher, 1914, p. 156). No adequate evidence has been offered to show that this megaspore tetrad has been derived directly from a more primitive ancestor of which this arrangement was typical, as it is of the megaspores of the higher pteridophytes. The same can be said of those other atypical angiosperms, having tetrahedrally arranged megaspore nuclei not separated by walls, such as *Gunnera* (Schnegg, 1902; Ernst, 1908; Samuels, 1912), the Penaeaceae (Stephens, 1909*a*) and certain *Euphorbias* (Modilewski, 1909). In none of these has the tetrahedral arrangement of the megaspore nuclei been shown to be primitive, and in each case this arrangement has been found within a rather rounded embryo sac.

4. *Embryo Sac.*—Under this head will be considered the disappearance of the megaspore walls, the further division of the megaspore nuclei, the formation of the egg apparatus, of the endosperm nucleus, and of the occasional peripheral cells.

The separating walls of the megaspores are delicate and do not persist long after their formation from the cell plates of the second division. As they disappear the cytoplasm again becomes continuous, and a large central vacuole replaces the series of numerous small peripheral vacuoles, characteristic of the earlier phases of development. This single continuous mass of protoplasm with its 4 nuclei must undoubtedly be regarded as the product of fusion of 4 megaspores, and the single individual embryo sac thus formed as a composite female gametophyte. This sort of composite young gametophyte has been observed in *Avena fatua* (Cannon, 1900) and in *Crucianella* (Lloyd, 1902), with the difference, however, that in these forms 3 of the megaspore nuclei degenerate, sometimes after one division each, while the fourth gives rise to all of the 8 nuclei of the mature embryo sac. Lloyd pointed out the significance of this association, of the first haploid nuclei from a megaspore mother cell, in one cell and spoke of this cell as "an individual by coalescence?" Coulter (1908) applied this same criterion, the chromosome number, to the interpretation of all embryo sacs in which the first nuclei produced in the ovule by the reduction division are included, either primarily or secondarily in a single protoplast. According to this view the embryo sacs not only of *Peperomia*, *Gunnera* and the Penaeaceae, but also those of *Lilium*, *Piper* and many others where the "megaspore mother cell develops directly into the embryo sac," are to be regarded as composite sacs. The demonstration of a reduction process and of evanescent megaspore walls in several species of *Peperomia* by Brown (1908) and Fisher (1914) and the especially clear case of tetrad formation in *P. hispidula* show that this genus furnishes several of the clearest instances of the formation of a composite mature embryo sac. The possibility of other interpretations of the observed phenomena which imply that chromosome reduction may occur at points in the life history, other than the divisions giving rise to the megaspore nuclei, have been suggested by Atkinson (1901), Brown (1908) and Ernst (1908, p. 27). The study of *P. hispidula*, however, together with that of other species by Brown and Fisher make it very clear that, in this genus, a genuine megaspore-formation is associated with the reduction division, and make it altogether probable that the same is true in *Piper*, *Lilium*, and in many other similar cases.

The 2 further divisions of each of the 4 megaspore nuclei are not followed by cell walls, nor even by cell plates. These facts suggest the homology of these divisions to the first steps of megaspore germination in ordinary angiosperms (see Brown, 1908, p. 453). Though it must be recalled that cell plates are formed in the first division of the megaspore nucleus of *Lilium*, *Clintonia* and perhaps others (R. W. Smith, 1911, p. 216). The 16 nuclei resulting from the 2 divisions of the megaspores are usually grouped in distinct quartets, one of them at the micropylar end of the embryo sac. Two of the nuclei of this micropylar group soon become surrounded by denser masses of cytoplasm and by cell walls, to form the egg and the single synergid. The other 2 nuclei of this group and the 12 of the other three groups later form a compact group near the middle of the short flask shaped embryo sac. This is the mature sac ready for fertilization. There are no 8-nucleate *mature* embryo sacs in this species as Coulter (1908, p. 363) has misunderstood me to state in my preliminary account of this species (Johnson, 1907). After fertilization, the occurrence of which is indicated by the presence of 2 nucleoli in the egg, the 14 nuclei of the central group fuse completely to form a huge, lobed, endosperm nucleus. Thus while the egg and synergid both come from one of the 4 megaspore nuclei the descendants of all 4 megaspores enter into the formation of the endosperm nucleus, making the latter the only really composite element in the embryo-sac.

From what has just been said it is evident that the striking peculiarities of the development of the embryo sac here are the occurrence of but 2 divisions of the megaspore nucleus instead of 3, the participation of all megaspores in the formation of a single sac and the organization of the endosperm nucleus by the fusion of so large a number of nuclei derived from megaspores. It seems perfectly clear, since the first 4 nuclei formed in the embryo sac are certainly megaspore nuclei, that the mode of megaspore germination here shown is not a primitive one but a specialized type reduced from one with 3 divisions such as occurs in other angiosperms. In the second place the single multinucleate prothallus or embryo sac formed by the cooperation of 4 megaspores has no analogue among lower seed plants or the pteridophytes, and therefore cannot possibly be regarded as primitive (see Campbell, 1899, p. 455, 1901, pp. 113-117, 1902, p. 781), or as recalling the many-celled prothallus of the gymnosperms and pteridophytes, which is everywhere developed from a single megaspore. Finally the formation of the endosperm nucleus by the fusion of 14 nuclei from 4 megaspores has no homologue outside of a few other peculiar angiosperms (*Gunnera*, Penaeaceae) and is pretty certainly a later specialization rather than a primitive type from which the typical endosperm nucleus, formed by two nuclei from a single megaspore, may have been derived. There is nothing about the development of this endosperm to suggest the possibility of its being a modified second embryo as suggested by Miss Sargant and others. It is to be recalled that Porsch has pointed out that no resemblance could be expected now, even if this were its nature. There is no evidence of the fusion of a second male nucleus in the endosperm group.

The occasional formation in *P. hispidula* of peripheral cells by the enclosure of one or two of the 16 nuclei of a sac by cell walls is probably to be regarded as a partial reversion to the fate characteristic of part of these nuclei in all of the 10 other *Peperomias* studied (Johnson, 1900*a*; Brown, 1908; Fisher, 1914). No instances of these peripherals were seen in my earlier work on *P. hispidula* (Johnson, 1907). The agreement of the other 10 species with each other is strong evidence that the embryo-sac with half a dozen peripheral cells is the more primitive type characteristic of the genus, from which the *P. hispidula* type, with few or none, has been derived.

There seems no particular reason for comparing the 1 or 2 peripheral cells of *P. hispidula*, when formed, or the 6 peripherals of other *Peperomias* with the antipodals of other angiosperms, except in so far as they are left over after the essential structures of the embryo sac have been organized. They certainly give no evidence of being sterile egg apparatuses, such as Porsch (1907) would expect to find in a primitive angiosperm.

Concerning the physiological cause and significance of the peculiar type of organization of the embryo-sac and endosperm nucleus found in *P. hispidula* we know nothing. Brown (1908), has suggested that the globular form of the mother cell gives all four of the megaspore nuclei formed in it an equal advantage in space and food supply. But it is to be remembered that other rounded mother cells may give rise to but one functional megaspore while in other angiosperms the composite embryo sacs are decidedly elongated. Among the latter are to be counted *Gunnera*, *Lilium*, and probably *Piper*, and many others with elongated sacs can be added to this list when the point at which chromosome reduction occurs has been determined.

Of the significance of the multiple fusion in the formation of the huge endosperm nucleus nothing definite can be said except to make the oft-repeated statement of fact that where two or more nuclei are left together in one protoplast they usually fuse to one nucleus. In the present instance the effect is to practically overcome the disparity in size of nucleus and protoplast (cf. figs. 58 and 88). The only evident physiological advantage of a fusion of the 14 nuclei into a single nucleus is that of forming a large nuclear unit adequate for the control of the large basal end of the embryo sac. Such a nucleus is perhaps especially needed for the formation of the relatively broad cell walls that are at first formed in the endosperm. Possibly also the activity of the endosperm cells in nourishing the embryo, during seed-development and at germination, is more efficient because of the presence of these large nuclei in them. But these advantageous *results* of the process do not, of course, explain its *causes* which will pretty certainly be determined only after experimental study.

5. *The development of the embryo and endosperm.*—The fertilized egg, with its two nucleoli, or its single enlarged nucleolus, remains undivided and unchanged, except for an increase in size, during the aggregation and fusion of the endosperm-forming nuclei and even during the first division of the fusion nucleus. Soon after this the oospore, or embryo, divides by a longitudinal wall and then by walls in other planes, whose sequence is undetermined, to finally form in the ripe seed, a suspensorless, slightly elongated embryo of about 50 cells.

The large, lobed, central endosperm nucleus has 20 or more nucleoli. It divides mitotically by a huge spindle whose axis is transverse to that of the sac and which has on it 144 or more chromosomes. The second and third series of walls in the endosperm are approximately longitudinal and these are followed by irregularly placed walls that form an ellipsoid mass of 100 or more cells in the ripe seed. The endosperm is cellular from the start, each mitosis from the first is accompanied by the formation of a cell plate and wall. The cells of the mature endosperm have large, lobed nuclei and rather dense cytoplasm, containing many oil globules but no starch.

This mode of development of the endosperm, in which a cell wall immediately follows each division of the nucleus, cannot be regarded as the most primitive type occurring among seed plants. I have already pointed out (Johnson, 1905, p. 31), that this mode of endosperm-formation is one not found at all among gymnosperms and occurs elsewhere among angiosperms only in forms that are generally recognized as rather highly specialized forms. This same point has been more fully elaborated by Samuelsson (1913, pp. 135–145). Moreover this *succedaneous* type of formation of endosperm cells is not encountered among pteridophytes, save in two highly specialized families, the Marsiliaceae and Salviniaceae, neither of which can be thought of as a very probable ancestor of angiosperms.

6. *Germination and the storage of food in the perisperm.*—At germi-

nation of the seed the endosperm, with the enclosed embryo, bursts out of the seed-coat and fruit and finally is itself ruptured by the radicle of the growing embryo. The tips of the cotyledons remain for a long time embedded in the cap of endosperm, which persists between them and the perisperm. From this position of the endosperm it must evidently serve as a conveyor of the stored starch from the perisperm to the cotyledons. This location of the stored starch in the sporangial tissue is by no means a very primitive feature among seed plants. It is found in no pteridophyte and in no gymnosperm, except the bare remnant of nucellus left in the seed of Torreya. Among angiosperms, both dicotyledons and monocotyledons, perisperm occurs only in forms that on other grounds have been considered decidedly specialized (Johnson, 1902, p. 337).

The persistence of the endosperm as a nurse for the embryo during its development and germination is characteristic of all the Piperales thus far studied and also of certain perisperm-containing monocotyledons (Johnson, 1900*b*, 1902). In the case of the orchids and the Podostemaceae, as described by Magnus (1913), endosperm is wanting and thus, as Magnus points out, the nutritive function of the female gametophyte is completely lost. In these two families this gametophyte serves simply to mature a functional egg apparatus. Magnus suggests that this disappearance of the endosperm allows a more rapid transfer of material to the embryo directly from the parent sporophyte, which is aided by a haustorium of the suspensor. It is not evident to the writer why this should allow more rapid nutrition of the embryo, nor is it proven that the embryos of these forms actually are developed more rapidly, in proportion to their size, than those of forms with endosperm. In the series of seed plants showing different degrees of development and functioning of the diploid generation, beginning with the gymnosperms and *Ricinus* and ending with the orchids and Podostemaceae, it is evident that *Saururus*, *Peperomia* and *Canna* show the next to the last step in the reduction of the endosperm, both in relative bulk and in variety of functions. Magnus suggests that such forms without endosperm, the Podostemaceae for example, may become the progenitors of entirely new races of seed plants. The haploid generation of these hypothetical plants might, we can imagine, come to consist merely of one of the four (or possibly three) haploid nuclei resulting from the reduction division of the nucleus of the megaspore mother-cell. It is also quite possible to conceive that one of these nuclei, without being cut off from the others by a cell wall, might be fertilized and so give rise to an embryo which should be nourished directly by the surrounding tissue of the parent diploid generation. If this happened we should have realized in the seed plants the extreme reduction of the diploid generation which is found in animals, and perhaps in Fucus, but which is only approximated by the Orchidaceae and Podostemaceae.

7. *The relative primitiveness of the 8-nucleate and 16-nucleate embryo-sacs.*—The view that the embryo-sacs with 16 nuclei are primitive, in that they retain more of the numerous nuclear divisions intervening between megaspore and ripe egg, which are characteristic of the gymnosperms and heterosporous pteridophytes, is one first advanced by Campbell (1900–1901). His conclusion was based on the assumption that all of the 16 nuclei present in the mature embryo sac result from the germination of a single megaspore. The recent work on several species of *Peperomia* (Johnson, 1907, Brown, 1908, Fisher, 1914), on the Penaeaceae (Stephens, 1909*a*), on *Gunnera* (Ernst, 1908, Samuels, 1912) and the present study show, as Coulter (1908, pp. 363–364) has suggested, that Campbell's assumption is clearly incorrect. The individual megaspore in each of these genera gives rise not to more prothallial nuclei than in other angiosperms but to just half as many, four instead of eight. It is clear that this can hardly be regarded as a very primitive mode of megaspore-germination. It is rather a more reduced type than the one usually found in angiosperms. From this point of view then Campbell's contention that the 16-nucleate embryo sac is primitive is clearly untenable. From this point of view also it is evident that, since the embryo sac of *Peperomia hispidula* is shown to be the product of division of 4 individual megaspore nuclei, in a common cell cavity, the number of nuclear divisions between the definitive archesporial cell and the mature embryo sac is not a matter of prime significance. Since then the reduction division and megaspore formation in *P. hispidula* goes on entirely in the normal way except for the arrangement of the spores in a tetrad, we cannot agree with Ernst (1908, p. 29), that the process of tetrad division here is reduded and that the germination of the megaspore involves one more division than usual and that this sac is thereby "*distinguished as an older, or at least as an independent, form of angiospermous embryo sac.*"

It would still, of course, be possible to regard the peculiar type of megaspore-germination found in *Lilium*, *Peperomia*, *Gunnera*, etc., as an independently developed one, if the forms possessing it were at all related genetically. It is a matter of fact, however, as has been pointed out before (Johnson, 1902, p. 336; Maneval, 1914, pp. 9, 10), that the forms possessing these composite sacs belong to widely separated families. Moreover this compound type of embryo sac may be found in but one or two genera of the family in which it occurs. Both these facts make it just as impossible to believe that the compound sac of *Lilium*, for example, is a long-established or fundamentally peculiar type as to believe that the very different peculiarities of the sac of *Tulipa sylvestris*, of *Trillium grandiflorum*, of *Clintonia borealis*, or of *Smilacina* are primitive and fundamentally peculiar in their significance. The same reasoning would also deter us from regarding the abnormalities of the sacs of *Cypripedium* (Pace, 1907), or *Epipactis* (Brown and Sharp, 1911), as other than recently acquired variations of the usual type of development of the angiospermous embryo sac.

Because of the scattered distribution of these abnormal types of embryo sac it seems quite clear that they have been independently developed. Moreover, if the 4-spored, 8-nucleate type of *Lilium* can be developed phylogenetically from the 1-spored, 8-nucleate type characteristic of its family as a whole, and therefore presumable primitive, then the 4-spored, 16-nucleate type of *Peperomia* may well have arisen from the 4-spored, 8-nucleate type of the other *Piperaceae*. That is, the 16-nucleate sac has in all probability, contrary to the opinion of Ernst, arisen from the 8-nucleate one (see Johnson, 1900*a*, 1902). For the same reason the single synergid of *Peperomia* must be regarded as a later, incidental modification of the usual arrangement, of no more significance than the fact that *Ornithogalum* has but one synergid while the rest of the Liliaceae have two.

Summary

1. In vegetative structure *Peperomia hispidula* is the simplest described species of the genus and family. The delicate herbaceous stem, the single closed bundle in stem and root, and the delicate, gland-covered leaf, are probably not primitive features but are due to recent modification of the more complex type of structure, which is characteristic of the other members of the genus.

2. This species has hermaphrodite, naked flowers and there is no indication in its development that it ever possessed floral envelopes. There are, as in other *Peperomias*, only 2 microsporangia per stamen

instead of the more primitive number, 4, that is found in most angiosperms. The ovary is of a single carpel, with no indication of the tricarpellate condition of which there seem to be clear traces in the development of its ally *Piper.*

3. The nucleus of the definitive archesporial cell has a thin, darkly staining concave disk lying just within its wall. This cell divides, with a characteristic synapsis and reduction in its nucleus, to 4 tetrahedral megaspores. The delicate walls of these megaspores soon disappear, leaving their 4 nuclei in a single, continuous protoplast. This globular tetrad of spores is probably a recent innovation in the development of this genus. It is perhaps a modification of the linear series of spores, characteristic of *Piper* and of most other angiosperms, that is related in some way to the globular form of the archesporial cell and sac of *Peperomia.* There is surely no adequate evidence that this tetrad arrangement has come down directly from a primitive ancestral form.

4. The four megaspore nuclei divide in the single protoplast to form one compound, 16-nucleate gametophyte, or embryo sac, consisting of an egg and a synergid, which are both from one megaspore, and of a huge endosperm nucleus formed by the fusion of the remaining 14 nuclei. This compound embryo sac has no ancestral fore-runner among gymnosperms or pteridophytes and is therefore regarded as a recently specialized type. It probably has arisen independently in that genus, for physiological reasons that have not yet been made clear. Quite rarely peripheral cells are formed, but only one or two, instead of the half dozen, characteristic of the other, probably more primitive, *Peperomias* that have been studied.

5. A cell wall immediately succeeds each division of the large endosperm nucleus and an endosperm is formed of 100 or more cells, containing some oil globules but no starch in the mature seed. This type of endosperm formation, which is found in no simpler plants nearer than the Marsiliaceae, cannot be regarded as primitive. Starch for the nutrition of the embryo is stored in the perisperm. This is probably not a primitive feature in angiosperms, since it does not occur in either gymnosperms or pteridophytes and is not known in any undoubtedly primitive angiosperm.

6. At germination the small, globular embryo is enclosed and nourished by the swelling endosperm until the primary organs of the former are organized. The tips of the cotyledons remain enclosed in

the endosperm until the starch of the perisperm is exhausted. This restriction of the functions of the endosperm to that of nurse for the embryo, is the next to the last step in the disappearance of the endosperm, which has become practically complete in the Orchidaceae, the Podostemaceae and the Helobiales.

7. The 16-nucleate embryo sac of *Peperomia* like those of other atypical angiosperms cannot be regarded as primitive. In the first place it is highly peculiar in being the product of four megaspores, germinating in a single protoplast, a phenomenon unknown among simpler forms. In the second place the angiosperms with 16-nucleate embryo sacs belong to isolated genera, in many unrelated families whose other genera have 8-nucleate sacs, from which it is believed the 16-nucleate sacs have become specialized independently in each family. Thirdly: the whole vegetative and reproductive structure and development of this species favor the view that it is a specialized form among its allies.

THE HARPSWELL LABORATORY,
August, 1914.

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EXPLANATION OF PLATES XLI–XLIII

FIG. 57. Sagittal section of ovary, ovule, and part of subtending bract. Showing initiation of stigma, stylar canal, parietal cell and definitive archesporial cell. ×90.

FIG. 58. Half-grown definitive archesporial cell (embryo sac mother-cell), showing character of chromatin net and cytoplasm. ×1,150.

FIG. 59. Similar section of slightly older embryo sac, showing incipient synapsis and the initiation of the nuclear disk. ×1,150.

FIG. 60. Similar section showing nearly complete synapsis. Nuclear disk distinct and free from nuclear wall. ×1,150.

FIG. 61. Similar section showing complete synapsis. Nuclear disk distinct. ×1,150.

FIG. 62. Similar section, showing double and twisted chromatin threads. Nuclear disk rough. (Nucleolus in an adjoining section.) ×1,150.

FIG. 63. Similar section of slightly later stage, showing formation of chromosomes. Nuclear disk not evident. ×1,150.

FIG. 64. Similar section of older embryo sac mother-cell, showing rounded chromatin masses in nucleus and large, darkly-staining granules in the cytoplasm, though nuclear wall is still intact. Nucleolus and nuclear disk not distinguishable. ×1,250.

FIG. 65. Similar section showing part of spindle of first division of megaspore mother-cell. The dark rods near the poles of the spindle are, perhaps, the remains of the nuclear disk. ×1,250.

FIG. 66. Part of a similar section, showing all chromosomes at a later phase of the same spindle. ×1,250.

FIG. 67. Similar section showing the chromatin in the partially organized nuclei resulting from the first mitosis in the embryo sac. Cell-plate evident. ×1,250.

FIG. 68. Approximately longitudinal section of ovule containing binucleate embryo sac, showing some remains of the spindle of preceding mitosis. The cell plate not distinguishable, probably because of the obliqueness of the section. ×350.

FIG. 69. Longitudinal section of embryo sac showing first 4 nuclei formed, with cell-plates. ×1,250.

FIG. 70. Similar section of an embryo sac, showing 3 megaspore nuclei surounded by cytoplasm and separated by cell walls, to form megaspores in a tetrad. ×1,250.

FIG. 71. The section adjoining that shown in last figure, showing the fourth nucleus and megaspore. ×1,250.

FIG. 72. Similar section of a more elongated embryo sac, with part of one of cell walls. ×600.

FIG. 73. Similar section of an embryo sac with 4 megaspore nuclei still connected by fibres. The central vacuole has increased in size. ×1,250.

FIG. 74. Similar longitudinal section of ovule with 4 unseparated megaspores. ×350.

FIG. 75. Similar section showing first mitosis of the 4 megaspore nuclei. ×665.

FIG. 76. Longitudinal section of an 8-nucleate embryo sac. ×665.

FIG. 77. Longitudinal section of 8-nucleate embryo sac with the nuclei in two groups. The nuclei shown in dotted outline are from the adjoining section of the same sac. ×530.

FIG. 78. Sagittal section of young fruit and bract showing vascular connection with axis. Embryo sac slightly diagrammatic. ×100.

FIG. 79. Transverse section of style, near middle of its length. Showing small-celled conducting tissue and large tracheae. ×125.

FIG. 80. Longitudinal section of 16-nucleate embryo sac, showing differentiation of cytoplasm about egg and synergid. The dotted nuclei are from an adjoining section. ×530.

FIG. 81. Similar section showing egg and synergid with walls. The nuclei of this sac are in 4 groups, showing their derivation from 4 megaspores. ×530.

FIG. 82. Similar section of older sac, with 14 prothallial nuclei grouped near center, ready for fusion. Dotted nuclei are from an adjoining section. Cytoplasm between vacuoles diagrammatic. ×530.

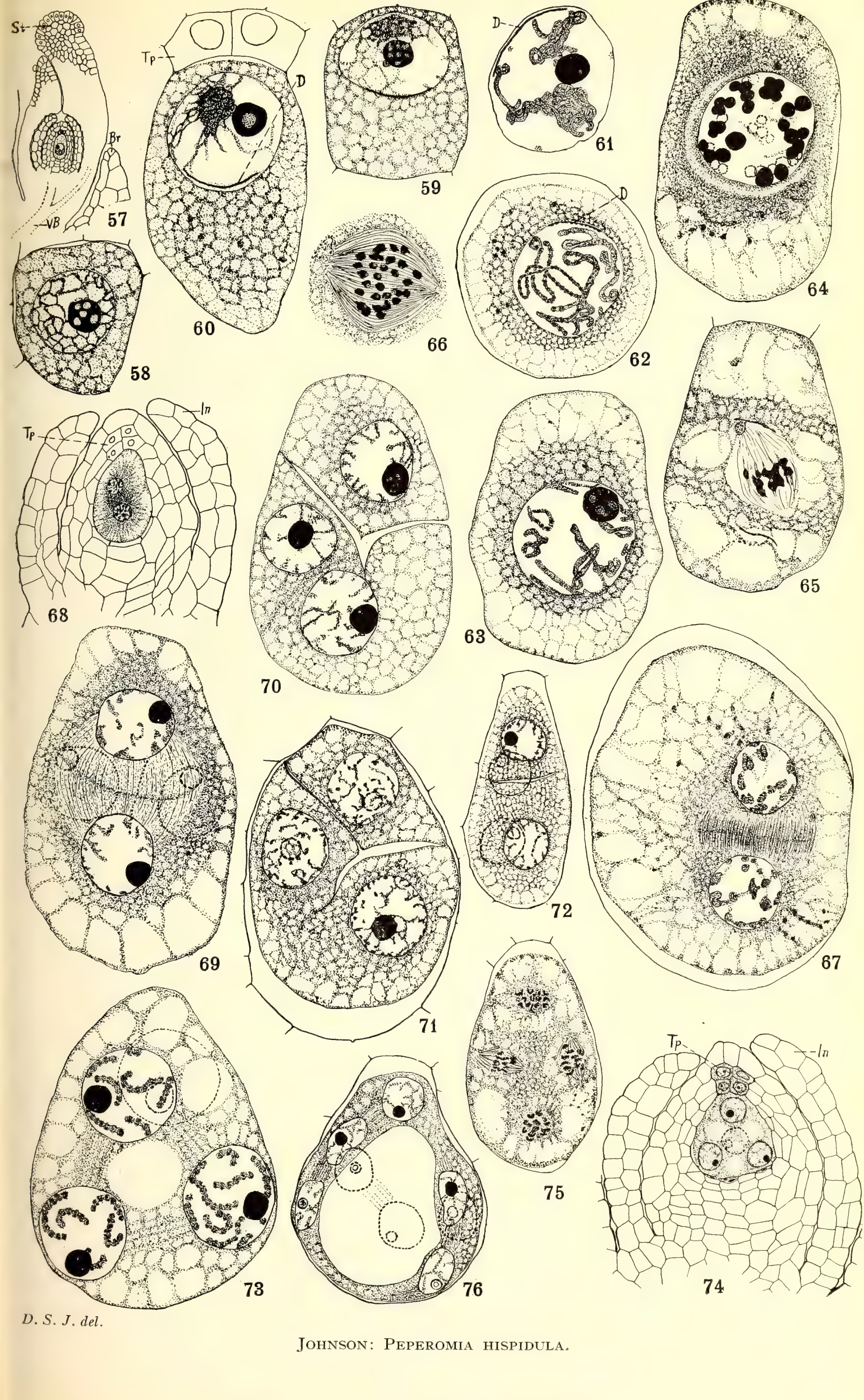
FIG. 83. Similar section of a sac in which fusion of the 14 constituents of the endosperm nucleus has begun, though they are still scattered around the central vacuole. The nucleoli of this and following figures are numbered in the order of their appearance in focus from one side of the sac to the other. Nucleoli 1 and 2 are in one fusion nucleus and 3 and 4 are in another. ×500.

FIG. 84. Similar section of an embryo sac, showing 13 grouped nuclei of which the large one, numbered 11, with a large nucleolus, has been formed by the fusion of 2 of the 14 free prothallial nuclei. The cytoplasm is becoming denser about the grouped nuclei. The 2 nucleoli in the egg indicate that fertilization has occurred. The small nuclei, 5 and 6, were perhaps destined to degenerate. ×900.

FIG. 85. Similar section showing 4 fusion nuclei in an endosperm group (those containing nucleoli 1–5). The nucleus at the right is enclosed in a peripheral cell against the wall of the sac. (Cytoplasm diagrammatic.) ×530.

FIG. 86. Basal half of similar section, showing more advanced stage of fusion. ×530.

FIG. 87. Longitudinal section of sac showing advanced stage of fusion of endosperm-forming nuclei, one lobe with 4 nucleoli. This sac is unique among those seen in having, in addition to the binucleolate egg nucleus and a synergid nucleus, two additional nuclei in the micropylar lobe. They may possibly have come from the pollen tube. The number of nuclei in the fusion mass could not be counted with certainty. ×530.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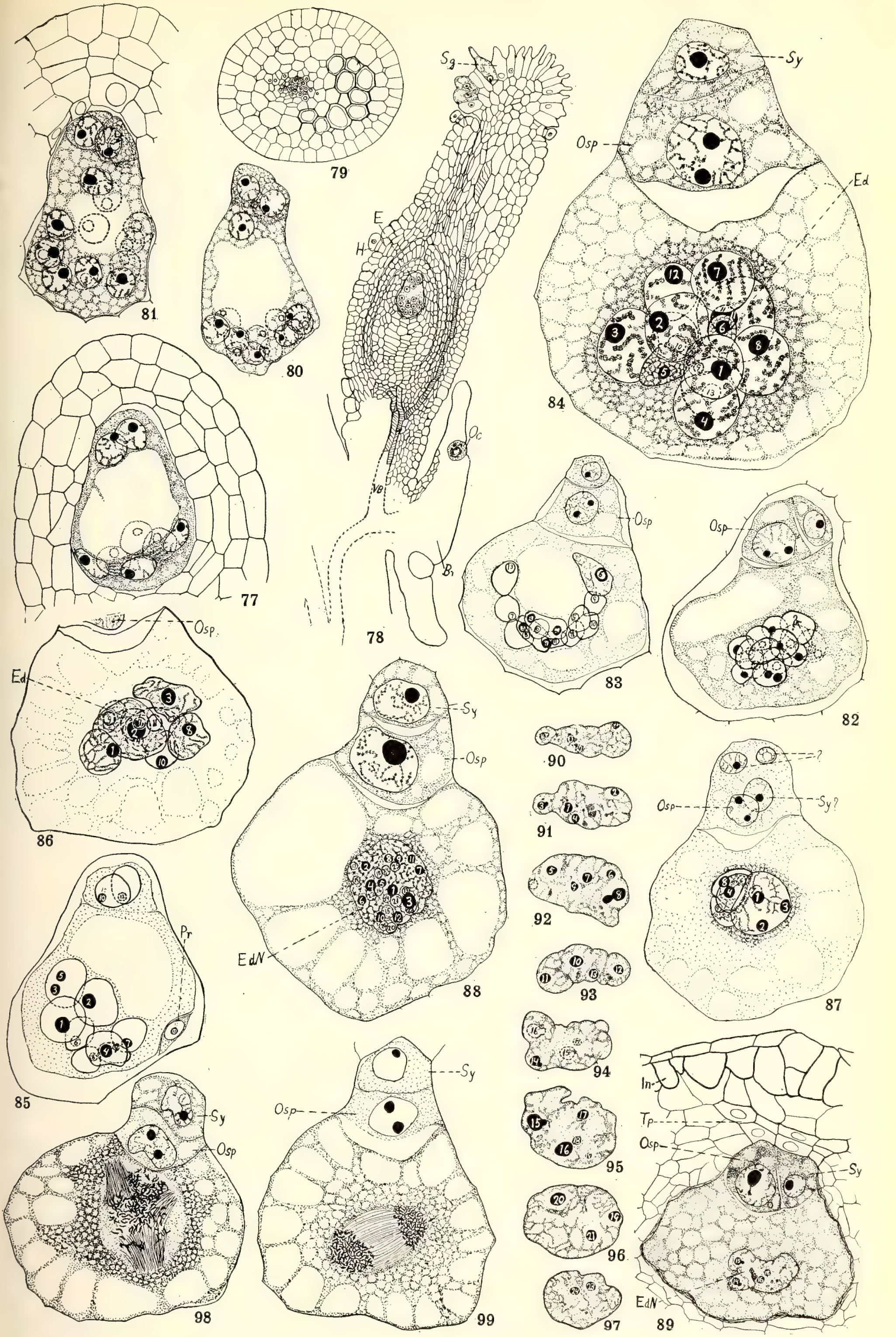
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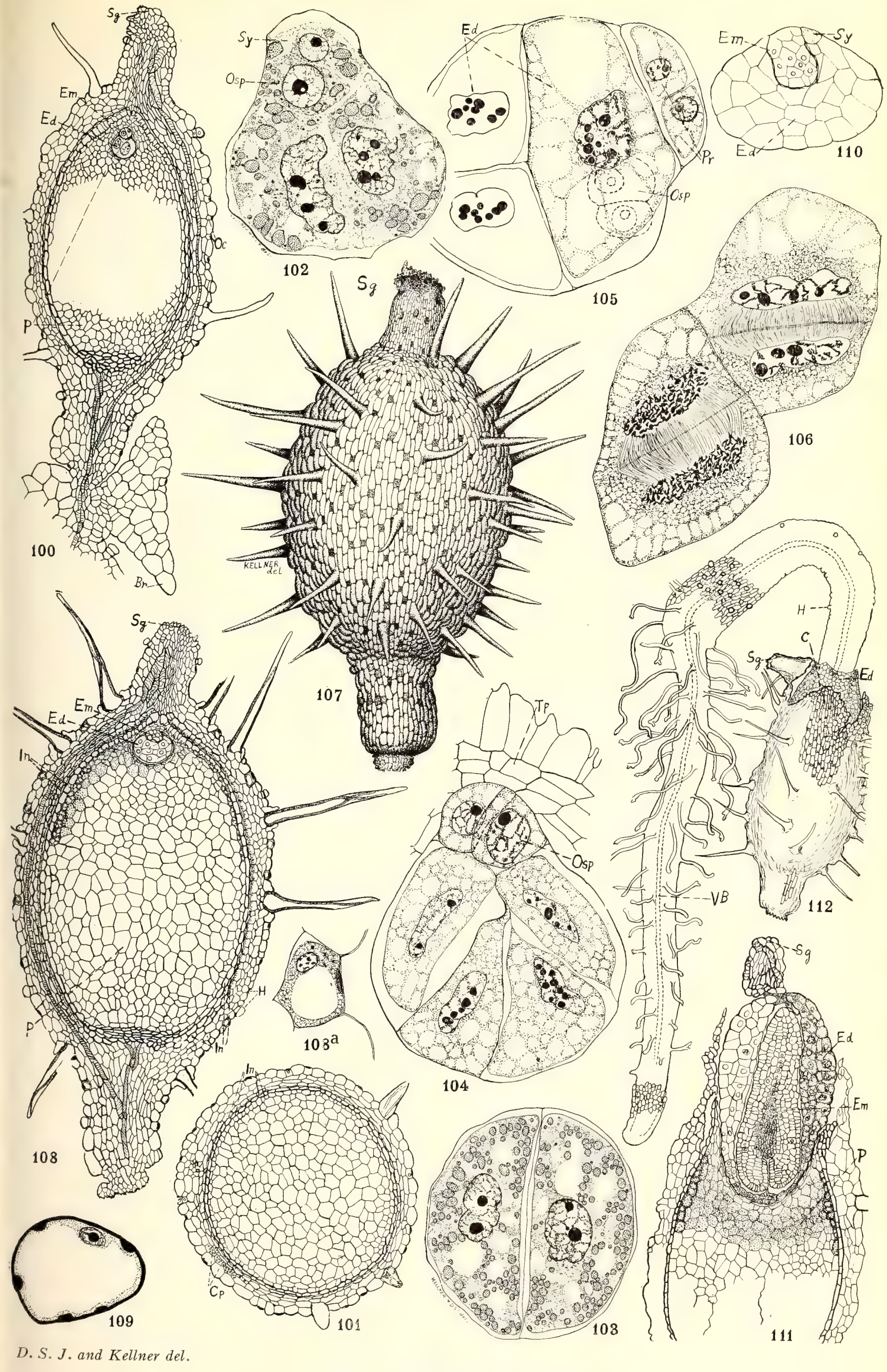
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JOHNSON: PEPEROMIA HISPIDULA.

FIG. 88. Similar section showing the single endosperm nucleus. Fusion is complete except for the lobing that indicates the composite origin of this nucleus. The nucleoli have already fragmented to form 26 nucleoli. Oospore with one double-sized nucleolus and a large, somewhat beaded chromatin net of apparently the same character throughout. ×530.

FIG. 89. Longitudinal section of an embryo sac and surrounding tissues, showing fertilized egg and one section of the single, much-lobed, endosperm nucleus. ×480.

FIGS. 90–97. Successive longitudinal sections of the same endosperm nucleus that is shown in Fig. 89, showing details of the lobing and the aggregation of the chromatin along the lines of fusion of the constituent nuclei. ×480.

FIG. 98. Longitudinal section of sac showing the oospore and the first mitotic spindle of the endosperm nucleus, with part of its very numerous chromosomes. ×530.

FIG. 99. Similar section showing later phase of first mitosis of endosperm nucleus. The spindle has the usual, transverse position. ×530.

FIG. 100. Sagittal section of a half-mature fruit and its bract, showing oospore, endosperm, perisperm, integument and carpel, with its bristles and single vascular bundle, on the abaxial side. ×55.

FIG. 101. Transverse section of a somewhat older fruit, showing perisperm, integument and carpel. ×55.

FIG. 102. Longitudinal section of embryo sac with oospore and 2 endosperm cells, showing the many nucleoli and the lobing of the endosperm nuclei. ×480.

FIG. 103. Transverse section of similar sac showing the first 2 endosperm cells, with their lobed nuclei. Numerous fat globules in the cytoplasm. ×480.

FIG. 104. Longitudinal section of sac with oospore and 4 endosperm cells. ×480.

FIG. 105. Transverse section of a sac with 3 endosperm cells having lobed, multinucleolate nuclei, and 2 peripheral cells with small nuclei having a single nucleolus each. The oospore and synergid (in dotted outline) are from an overlying section. ×480.

FIG. 106. Part of a longitudinal section of an embryo sac showing the numerous chromosomes of the endosperm nucleus and two stages in the cell plate that is formed at each division of the latter. ×1,160.

FIG. 107. Lateral view of surface of mature fruit. ×55.

FIG. 108. Sagittal section of nearly mature fruit. ×55.

FIG. 108*a*. Part of the same section showing structure of an oil-cell. ×520.

FIG. 109. Transverse section of bristle of nearly mature fruit, showing the internal, slightly spiral thickenings. ×480.

FIG. 110. Longitudinal section of a mature embryo, endosperm and synergid. ×225.

FIG. 111. Longitudinal section of germinating seed, showing elongated embryo still enclosed by the swollen, active endosperm. The upper part of the carpel is pushed off by the endosperm. The perisperm is partially exhausted of starch near the endosperm. ×55.

FIG. 112. Surface view of young seedling with cotyledons still embedded in the endosperm. (From a glycerine mount.) ×25.

ON THE CORRELATION BETWEEN SOMATIC CHARACTERS AND FERTILITY. II.

ILLUSTRATIONS FROM PHASEOLUS VULGARIS

J. Arthur Harris

I. Introductory Remarks

The purpose of this paper, like that of the one which preceded it,[1] is the presentation of quantitative data toward the solution of the relationship between the degree of development of the somatic organs of the plant individual and its fertility.

The materials are drawn exclusively from pedigreed dwarf varieties of garden beans, *Phaseolus vulgaris*. These varieties are being described in biometric terms elsewhere, as shown in the bibliography; hence it is only necessary to designate the series by the key letters which open the detailed information of other papers.

The characters here treated are number of pods per plant, number of ovules formed per pod and number of seeds matured per pod.

II. Analysis of Data

Table I shows the correlations, r_{po}, r_{ps}, and the partial correlation ${}_{o}r_{ps}$, when p = pods per plant, o = ovules per pod, s = seeds per pod. The actual number of plants and the number of pods counted are also entered. Because of the great bulkiness of the data it is not feasible to publish even in condensed form the 64 correlation tables from which these constants were deduced.[2]

The correlations of this table are also shown in diagram 1.

I turn first to the question of linearity of regression. This is essential statistically, since upon the distribution of the means of the arrays of ovules or seeds in a sensibly straight line depends the va-

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[1] Biometrika 8: 52–65. 1911.

[2] The number of pods per plant was weighted with the number of pods counted in calculating mean and standard deviation of number of pods. Sheppard's modification was nowhere applied, variation being by discrete units. The number of pods in which the number of ovules and seeds was determined was used as n in the calculation of the probable errors.

TABLE I
CONSTANTS FOR VARIOUS SERIES OF BEANS

Series	Number of Plants	Number of Pods Examined	Correlation: Pods per Plant and Ovules per Pod	$\frac{r_{po}}{Er_{po}}$	Correlation: Pods per Plant and Seeds per Pod	$\frac{r_{ps}}{Er_{ps}}$	$r_{ps}-r_{po}$ and Probable Error	$\frac{r_{ps}-r_{po}}{E}$	Partial Correlation ${}_o r_{ps}$	$\frac{{}_o r_{ps}}{E_o r_{ps}}$
L	185	1,804	.040 ± .016	2.50	−.022 ± .016	− 1.39	−.061 ± .022	− 2.75	−.040 ± .016	− 2.50
LL	1,141	8,043	.068 ± .008	9.11	+.050 ± .008	+ 6.72	−.018 ± .011	− 1.70	+.028 ± .008	+ 3.72
LG	182	806	.230 ± .022	13.87	−.046 ± .024	− 1.93	−.345 ± .032	−10.79	−.082 ± .024	− 3.48
GG	747	6,310	.023 ± .008	2.76	−.028 ± .008	− 3.35	−.051 ± .012	− 4.35	−.035 ± .008	− 4.12
GGH	583	5,251	.127 ± .009	13.93	+.135 ± .009	+14.88	+.009 ± .013	+ .67	+.087 ± .009	+ 9.47
GGH_2	499	3,502	.066 ± .011	5.81	+.104 ± .010	+10.28	+.038 ± .015	+ 2.52	+.082 ± .011	+ 7.23
GGHH	396	2,656	.097 ± .013	7.51	+.096 ± .012	+ 8.06	−.001 ± .018	− .06	+.056 ± .013	+ 4.29
GGD	514	1,438	.124 ± .018	7.09	+.022 ± .018	+ 1.27	−.102 ± .025	− 4.10	−.024 ± .018	− 1.37
GGD_2	449	1,227	.124 ± .019	6.57	+.090 ± .019	+ 4.72	−.035 ± .027	− 1.30	+.041 ± .019	+ 2.13
GGDD	342	807	.074 ± .024	3.14	+.105 ± .021	+ 4.96	+.031 ± .032	+ .97	+.083 ± .024	+ 3.51
H	379	5,141	.200 ± .009	22.22	+.077 ± .009	+ 8.21	−.123 ± .013	− 9.45	−.013 ± .009	− 1.41
HH	1,484	14,029	.263 ± .005	49.58	+.325 ± .005	+64.96	+.062 ± .007	+ 8.61	+.230 ± .005	+42.60
HHH	1,271	11,230	.203 ± .006	33.20	+.206 ± .006	+34.33	+.004 ± .009	+ .41	+.135 ± .006	+21.68
HD	1,416	5,581	.170 ± .009	19.48	+.019 ± .009	+ 2.06	−.151 ± .012	−12.18	−.058 ± .009	− 6.50
HDD	1,204	5,449	.275 ± .008	32.71	+.161 ± .009	+18.04	−.114 ± .012	− 9.36	+.028 ± .009	+ 3.06
D	550	1,473	.355 ± .015	23.20	+.150 ± .017	+ 8.75	−.205 ± .023	− 8.97	−.044 ± .018	− 2.54
DD	513	1,827	.320 ± .014	22.67	+.151 ± .015	+ 9.83	−.168 ± .021	− 8.10	−.009 ± .016	− .57
DDD	459	2,018	.203 ± .014	14.18	+.185 ± .014	+12.88	−.017 ± .020	− .86	+.109 ± .015	+ 7.34
DH	670	5,955	.310 ± .008	39.29	+.338 ± .008	+43.92	+.028 ± .011	+ 2.52	+.211 ± .008	+25.33
DHH	565	5,019	.243 ± .009	27.29	+.228 ± .009	+25.33	−.015 ± .013	− 1.18	+.138 ± .009	+14.76
USC	530	2,569	.181 ± .013	14.14	+.055 ± .013	+ 4.23	−.126 ± .018	− 6.98	−.006 ± .013	− .43
USS	680	6,605	.151 ± .008	18.20	+.113 ± .008	+13.63	−.038 ± .012	− 3.25	+.043 ± .008	+ 5.22
USH	361	3,406	.242 ± .010	22.44	+.137 ± .011	+12.15	−.105 ± .016	− 6.74	+.027 ± .012	+ 2.37
USHH	224	1,743	.112 ± .016	7.03	+.010 ± .016	+ 6.26	−.012 ± .022	− .54	+.063 ± .016	+ 3.94
USD	312	802	.160 ± .023	6.91	+.028 ± .024	+ 1.18	−.132 ± .033	− 3.98	−.035 ± .024	− 1.48
USDD	237	851	.319 ± .021	15.41	+.187 ± .022	+ 8.39	−.132 ± .030	− 4.34	+.079 ± .023	+ 3.43
FSC	586	2,876	.206 ± .012	17.15	+.112 ± .012	+ 9.04	−.094 ± .017	− 5.45	+.045 ± .013	+ 3.58
FSS	868	7,809	.204 ± .007	27.88	+.226 ± .007	+31.42	+.023 ± .010	+ 2.23	+.166 ± .007	+22.43
FSH	475	4,541	.316 ± .009	35.12	+.251 ± .009	+26.68	−.065 ± .013	− 5.02	+.126 ± .010	+12.76
FSHH	427	3,837	.230 ± .010	22.29	+.197 ± .011	+18.71	−.033 ± .015	− 2.27	+.117 ± .011	+10.97
FSD	428	1,449	.265 ± .016	16.16	+.128 ± .017	+ 7.36	−.137 ± .024	− 5.76	+.015 ± .018	+ .87
FSDD	387	1,556	.267 ± .016	16.87	+.165 ± .017	+ 9.94	−.102 ± .023	− 4.44	+.060 ± .017	+ 3.55

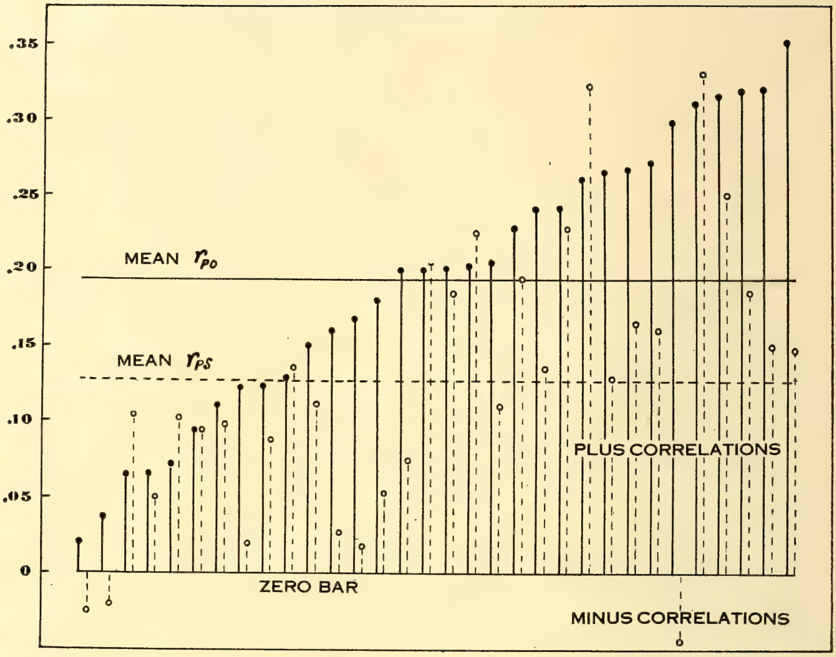

DIAGRAM 1. Magnitudes of correlations r_{po} and r_{ps} in 32 series of garden beans. The series are arranged in order of magnitude of r_{po}: r_{po} = solid lines and dots; r_{ps} = broken lines and circles; $\bar{r}_{po}$ = solid bar; $\bar{r}_{ps}$ = broken bar.

lidity of r as a measure of interdependence. It is of interest and importance biologically, since it shows whether the rate of modification of an associated character changes from one end to the other of the range of variation of the independent variable.

The labor involved in testing graphically the agreement of theoretical and empirical means for all of the series is prohibitive. Other methods of dealing with the problem are, as will be clear in a moment, unfavorable. I have, therefore, contented myself with the graphical treatment of a few illustrative cases (table II and diagrams 2 and 3).

In the graphs the means for the full range of variation in number of pods per plant has not been shown.[3] This arises from the fact that

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[3] The observed range of variation in number of pods per plant is indicated in the table. Limitations of space preclude the drawing of a scale for the means of ovules and seeds for each line, but it is the same for all and may be estimated fairly well by the reader from the end points of the lines as given in the table.

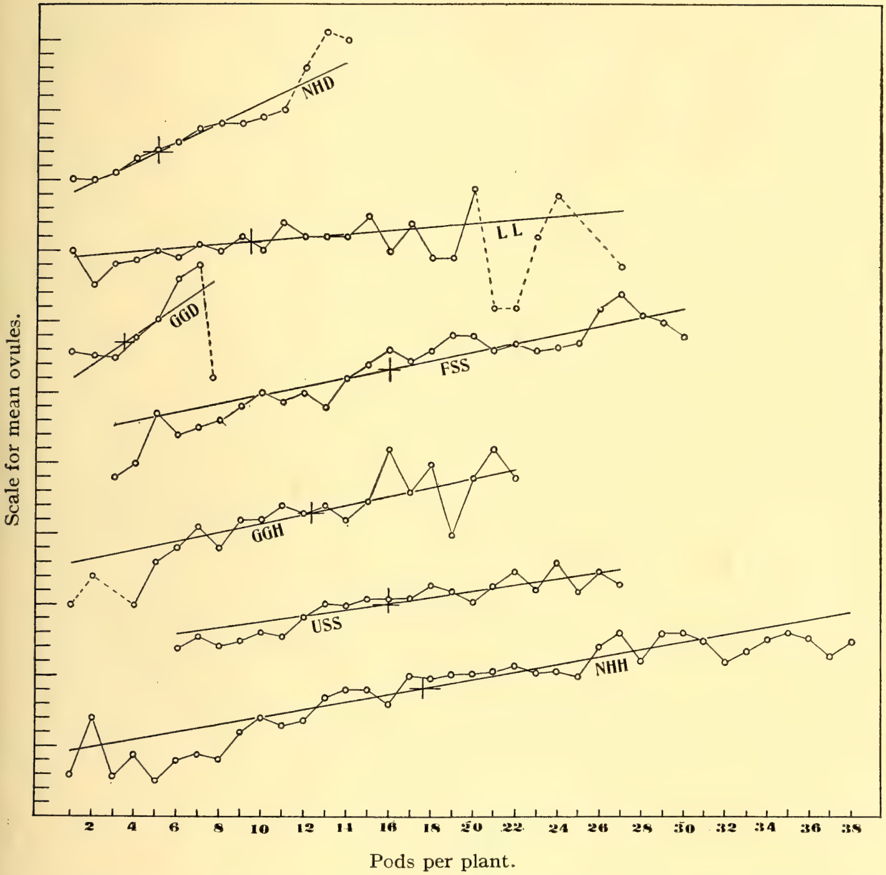

Diagram 2. Regression of ovules per pod on number of pods per plant. The end points of the lines are given in the table.

variation in the number of pods is very great[4] and that in consequence the extreme cases are represented by very few individuals. Thus in the HH series the number of pods per plant ranges from 1 to 67, but of the

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[4] The coefficients of variation for the number of pods per plant (unweighted) for the series illustrated in the graphs are:

HH = 51.02
HD = 48.97
GGH = 38.60
USS = 38.36

FSS = 49.34
GGD = 44.07
LL = 58.55
Mean = 46.99

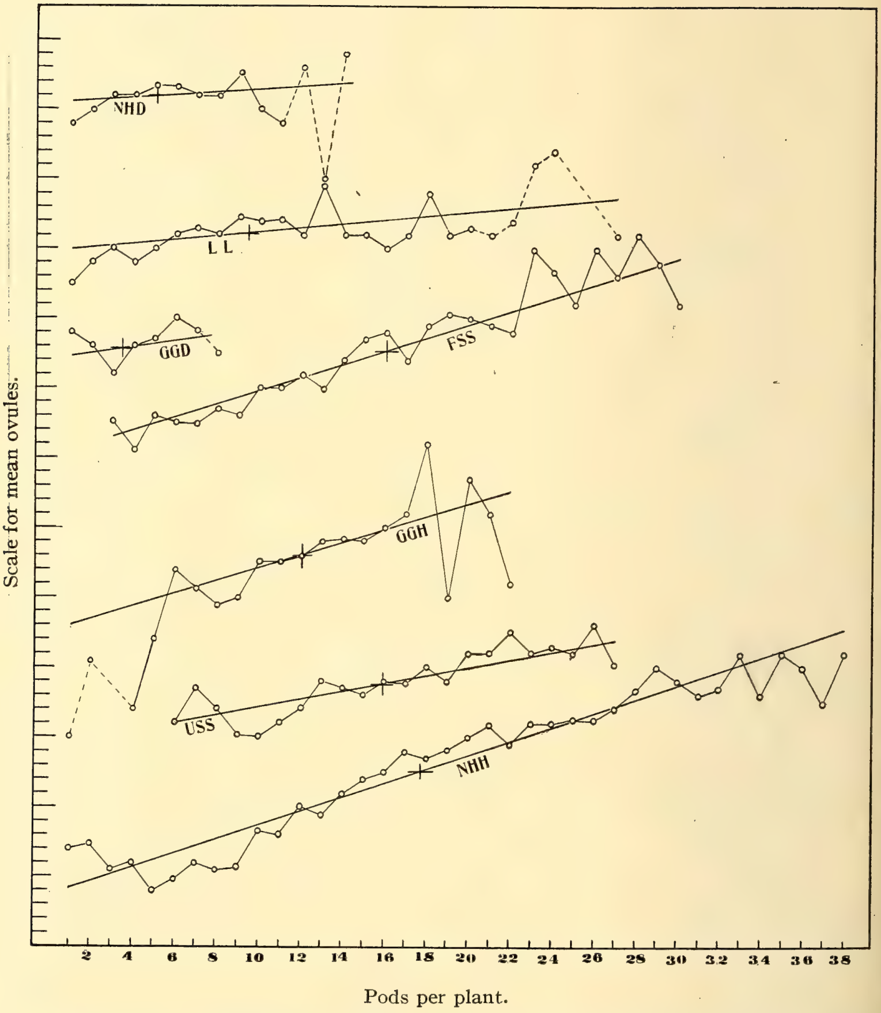


DIAGRAM 3. Regression of seeds matured per pod on number of pods per plant.

1,484 plants there are only 36 which produce more than 38 pods. To calculate means for arrays of such sizes is idle. Neither is it desirable, in view of the great differences in the range of variation from series to series, to combine classes in order to secure larger arrays. Any objections based on the fewness of individuals in the arrays applicable to

TABLE II

EQUATIONS FOR REGRESSION OF OVULES PER POD AND SEEDS PER POD ON NUMBER OF PODS PER PLANT

Series	Regression Equation	End Points of Line	Range of Pods
HH	$o = 4.940 + .027\,p$	1 = 4.97, 38 = 5.96	1–67
HH	$s = 3.388 + .050\,p$	1 = 3.44, 38 = 5.28	1–67
HD	$o = 4.375 + .070\,p$	1 = 4.44, 14 = 5.35	1–14
HD	$s = 3.072 + .011\,p$	1 = 3.08, 14 = 3.23	1–14
GGH	$o = 5.300 + .031\,p$	1 = 5.33, 22 = 5.99	1–33
GGH	$s = 3.743 + .046\,p$	1 = 3.79, 22 = 4.76	1–33
USS	$o = 5.173 + .022\,p$	6 = 5.31, 27 = 5.77	1–50
USS	$s = 3.457 + .026\,p$	6 = 3.62, 27 = 4.17	1–50
FSS	$o = 5.202 + .030\,p$	3 = 5.23, 30 = 6.11	1–54
FSS	$s = 2.536 + .047\,p$	3 = 2.58, 30 = 3.95	1–54
GGD	$o = 4.561 + .089\,p$	1 = 4.65, 8 = 5.27	1–8
GGD	$s = 2.709 + .022\,p$	1 = 2.73, 8 = 2.88	1–8
LL	$o = 4.950 + .013\,p$	1 = 4.96, 27 = 5.30	1–27
LL	$s = 2.515 + .014\,p$	1 = 2.53, 27 = 2.88	1–27

graphical methods of testing for linearity are equally valid, or even stronger, for formal tests, for example those based on a comparison of r and η. For 0 correlation η may have a substantial value when the arrays are numerous; it is in just such cases as this that very misleading results might be obtained through a careless application of Blakeman's test.

In the graphs the theoretical lines are drawn according to the equations calculated from the whole material, but are not extended beyond classes of pods per plant represented by only five individual plants or fewer.

The slope of all the lines is slight and the scatter of the empirical means decidedly large. This is precisely the condition to be expected in widely varying and but slightly correlated characters. For the benefit of those who may not be familiar with either the biological or the statistical phases of the study it may be pointed out that the irregularities seen in these diagrams are neither due to carelessness in the biological experiments nor to inadequacies of the statical methods, but solely to the difficulties inherent in the material.

With regard to the critical question, that of the sensible linearity or non-linearity of the empirical means, the diagrams do not—and largely because of this great irregularity—furnish a final answer. But while there is some indication of significant non-linearity, for practical purposes a straight line describes the change in o and s associated with an increase in p with sufficient accuracy.

Having justified the use of r[5] as a constant describing the degree of interdependence between p and o and s, I turn to the constants themselves.

The correlations are conspicuous for their irregularity. Practically speaking, however, they are positive throughout. This is strictly true for the relationship between pods per plant and ovules per pod. It holds for 29 of the 32 measures of the relationship between pods per plant and seeds per pod; of the 3 negative correlations all are small and neither can be safely regarded as statistically trustworthy with regard to its probable error.

Computing the physical constants of these varying values of correlation I find

	r_{po}	r_{ps}
Mean	.1948 ± .0107	.1264 ± .0109
Standard deviation	.0899 ± .0075	.0915 ± .0077
Coefficient of variation	46.16	72.38

The relationship between number of pods and ovules per pod is apparently higher than that between the number of pods and the number of seeds matured. The first of these may be considered a more strictly morphogenetic relationship, the second a more truly physiological one. The difference, .0684 ± .0153, is nearly four and a half times its probable error, and so perhaps significant.

Further evidence may be obtained upon the problem of the relative values of the two correlations by considering the differences in the two constants for each experiment separately, as shown in table I. In 7 cases $r_{ps} > r_{po}$, but in 25 cases $r_{po} > r_{ps}$. Taking the ratio of the differences to their probable errors, it appears that 3 of the cases in which the correlation for seeds is higher than that for ovules may possibly be considered significant (> 2.5) while there are 18 cases in which the correlation for ovules is higher that may be taken to be trustworthy statistically. The mean value of the ratios of the positive differences, $r_{ps} > r_{po}$, to their probable errors is 2.56, while that for the negative differences is 4.96.

It is, therefore, clearly demonstrated that although the values of the correlations are both low and irregular, those measuring the relationship between the number of pods and the number of ovules are sensibly higher than those measuring the relationship between the number of pods and the number of seeds matured per pod.

[5] Even if regression is not strictly linear, r is the best constant to be used, since η would be too largely affected by the errors of sampling in the numerous small arrays.

There can be little doubt that the observed statistical correlation between the number of pods per plant and the number of ovules laid down per pod describes a real morphogenetic interdependence. The constants further show that the relationship for the particular characters under consideration is not strict but lax.

The biological interpretation of the numerical values for number of pods and number of seeds matured per pod is not so simple. Here a second set of innate and environmental physiological factors—those involved in determining whether a given ovule shall die or develop—are superimposed upon the morphogenetic and physiological complex involved in determining the degree of development of p and o.

The most patent effect of these superimposed physiological factors is the death (non-development) of a considerable proportion of the ovules. The number failing to develop into seeds varies widely from pod to pod, but on the average it is roughly, though as I have shown (1913*e*) not exactly proportional to the number of ovules per pod. As a result there is a correlation of medium intensity between the number of seeds developing per pod and number of ovules formed (1912*d*).

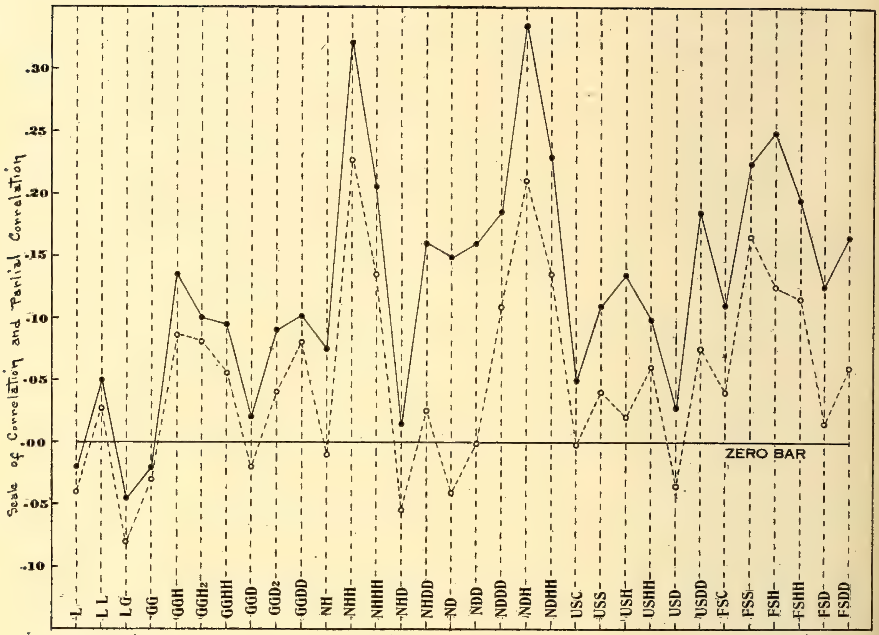
Clearly, therefore, there may be (at least) two quite independent sets of factors influencing the correlation between p and s. The first of these is involved in the interdependences r_{po} and r_{os}; as a resultant of these forces some relationship must exist between p and s. It is conceivable that this correlation which indicates no direct and independent physiological or morphogenetic nexus between p and s may account for the whole of the observed value of r_{ps}.

To correct for the influence of r_{po} and r_{os} on r_{ps}, I have recourse to the partial correlations between pods per plant and seeds per pod for constant numbers of ovules per pod. These have been calculated from the usual formula

$$_or_{ps} = \frac{r_{ps} - r_{po}r_{os}}{\sqrt{1 - r_{po}^2}\sqrt{1 - r_{os}^2}}$$

for each of the series. The values for r_{po}, r_{ps} are of course those of this paper. Those for r_{os} have already been published (1912*d*).

The values, table I, are also shown graphically in comparison with the correlations r_{ps} in diagram 4. The reduction in the intensity of the relationship between number of pods and number of seeds when correction is made for the relationship of these two characters with number of ovules per pod is clearly marked: it occurs in every in-



Series of plants.

DIAGRAM 4. Comparison of the correlations r_{ps} (solid dots and firm line) and the partial correlations ${}_o r_{ps}$ (circles and broken line) for individual series.

dividual case; only 3 of the values of r_{ps} have the negative sign while 10 of the partial correlation coefficients, ${}_o r_{ps}$, fall below the zero bar: $\bar{r}_{ps} = .1264 \pm .0109$, ${}_o\bar{r}_{ps} = .0507 \pm .0090$.

Notwithstanding the material reduction in r_{ps} when correction is made for the influence of r_{po}, r_{os} there seems to be a residuum of interdependence which cannot be thus accounted for. Evidence for this conclusion is seen in four facts. (*a*) The mean partial correlation has a positive and perhaps significantly positive value: ${}_o\bar{r}_{ps} = .0507 \pm .0090$, ${}_o\bar{r}_{ps}/E\ {}_o\bar{r}_{ps} = 5.64$. (*b*) Of the individual values of ${}_o r_{ps}$ only 10 of the 32 are negative in sign. (*c*) The mean value of the negative coefficients is only $-.0347$ while that of the positive constants is $+.0895$. (*d*) Of the 10 negative coefficients 5 may perhaps be considered statistically significant with regard to their probable error, although this number would be reduced if n were taken as plants instead of pods in the calculation of the probable error. Of the positive coefficients 19 are over 2.5 times their probable error, as compared with the 5 possibly significant values noted for the negative. Furthermore, the mean of the ratios of the 10 negative coefficients to their probable error is only 2.49 as compared with a mean ratio of 9.74 for the positive coefficients.

Thus the evidence seems to justify fully the assertion that there is a slight physiological relationship between the number of pods which a plant produces and the number of seeds which it is capable of maturing in these pods, and that this relationship is quantitatively independent of the more strictly morphogenetic factors linking together number of pods and number of ovules per pod.

Further than this the analysis cannot safely be pushed on the basis of available data.

III. Comparison of the Foregoing Constants with other Correlations for Somatic Characters and fertility

Since many additional series of data bearing on these problems are in hand and will eventually be published, I shall not compare in detail the results set forth here with others but I shall indicate merely the order of correlations which have been found.

For length of flowering stalk and number of flowers in the umbel-like inflorescence of two liliaceous plants the correlations are roundly[6]

For *Nothoscordium striatum*, $r = .500$,

For *Allium stellatum*, $r = .620$.

For the relationship between the length of the flowering stalk (pedicel) and number of ovules and seeds per fruit the only available data are[7]

For *Sanguinaria*, $r_{lo} = .323 \pm .019$,

For *Sanguinaria*, $r_{ls} = .363 \pm .019$.

Here $r_{ls} > r_{lo}$, but $r_{ls} - r_{lo} = .040 \pm .027$ only. Thus the difference is hardly significant with regard to its probable error; there is only a single series of material; further collections might show that here, as in garden beans, the correlation for degree of vegetative development and number of seeds is on the average lower than that for vegetative development and number of ovules per pod. The partial correlation in the case of *Sanguinaria* would of course have a significantly positive value.

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[6] Ann. Rept. Mo. Bot. Gard. **20**: 105–115. 1909.

[7] Biometrika **7**: 316. 1910.

In these three cases the bulb or rootstock sends up only a single inflorescence. The correlation for length and ovules and seeds in *Sanguinaria* is much closer to the r_{po} and r_{ps} in beans than are the correlations in *Nothoscordium* and *Allium*. This is perhaps to be expected, since in the two Liliaceae the correlation is between the length of a main axis and the number of secondary axes originating from it, while in *Sanguinaria* and in the beans the correlation is (roughly speaking) between the degree of development of the axis and the characteristics of the ovaries which it produces.

That the correlations in *Sanguinaria* are sensibly higher than the average values for beans may be due in part to chance—a few of the values for beans reaching almost the magnitude of those found in the blood-root. It is perhaps more probable that the higher value in *Sanguinaria* is due to the facts (*a*) that the correlation is between two intimately associated organs—a single fruit terminating the simple axis the length of which furnishes the measure of the degree of somatic development, whereas in garden beans the axis is extensively divided—and (*b*) that *Sanguinaria* is a perennial and there is doubtless considerable age heterogeneity which probably tends to raise the correlation.

If one extends the range of comparison to include the relationships within inflorescences there are several available constants.

In *Crinum longifolium*[8] I have found for fruits per inflorescence and seeds per fruit, $r_{fs} = -.072 \pm .024$. For the inflorescence of *Celastrus scandens*[9] I have shown

For flowers and seeds, $r = .033 \pm .013$,

For fruits and seeds, $r = -.012 \pm .013$.

In *Staphylea* the correlation between number of fruits per inflorescence and number of ovules and seeds per pod has been investigated[10] for series of individual shrubs, as well as for random collections from numbers of shrubs. It is difficult in these cases to be sure of even the sign of the correlation. The correlation for number of fruits and length of fruit is low.[11] Those for number of fruits and radial asymmetry and locular composition are also very small.[12]

For three series of intact inflorescences from individual trees of the legume *Cercis canadensis* in which the number of ovules in the ovaries was determined by clearing with alcohol and counting under the microscope, the results are[13]

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[8] Ann. Rept. Mo. Bot. Gard. **23**: 89–91. 1912.

[9] Ann. Rept. Mo. Bot. Gard. **20**: 120–122. 1909.

[10] Beih. Bot. Centralbl., Abt. 1, **28**: 2–10. 1911.

[11] Bot. Gaz. **53**: 401–402. 1912.

[12] Zeitschr. Ind. Abst.- u. Vererbungslehre **5**: 183–187. 1911.

Tree 1, $r = -.007 \pm .023$,
Tree 2, $r = .030 \pm .021$,
Tree 3, $r = .134 \pm .024$,

where the probable errors are calculated on the basis of the number of ovaries dissected out and examined, not the number of inflorescences.

Thus the correlations between the number of flowers formed or number of fruits matured per inflorescence and the fertility of the fruits—either as measured by the number of ovules laid down or the number of seeds ripened—are without exception very low indeed.

IV. Summary and Discussion

This paper, which is one of a series on the various aspects of fertility and fecundity in plants, is a contribution of data towards the solution of the problem of the relationship between the degree of somatic development of the plant individual as measured by the number of fruits which it bears, and the fertility of these fruits as measured by the number of ovules formed and the number of seeds developing.

The data comprise the records of number of ovules formed and number of seeds matured in 127,610 pods of garden beans, *Phaseolus vulgaris*, from 19,064 plants with known number of pods drawn from 32 cultures made under a wide range of environmental conditions and embracing five different varieties.

Taken in connection with data recorded in other papers they permit the consideration of points not touched upon here. It is perhaps fair to state that these are to be discussed in subsequent papers where extensive additional series of records already in hand can be reduced.

The analyses of the data as far as carried out lead to the folowing conclusions:

(1) The correlation between number of pods per plant and number of ovules per pod has always been found positive but low, ranging from .023 to .355, with an average value of about $\bar{r}_{po} = .195$.

(2) For pods per plant and seeds per pod the correlations are also for the most part positive, although constants which have the negative sign but are insignificant with regard to their probable errors sometimes occur.

These values range from—.046 to .338 with a mean of $\bar{r}_{ps} = .126$.

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[13] Bot. Gaz. 53: 403–404. 1912.

By all available tests the coefficients for pods per plant and ovules per pod seem to be significantly higher than those for pods per plant and seeds per pod.

(3) As a resultant of the relationship between pods per plant and ovules per pod (r_{po}) and that demonstrated elsewhere between ovules and seeds per pod (r_{os}) some correlation must be expected between number of pods per plant and number of seeds per pod, whether there be any direct physiological interdependence between these two characters or not.

(4) By determining the correlation between p and s for constant values of $_{o}$, *i. e.* by calculating $_{o}r_{ps}$ by the usual partial correlation formulae for three variables, I have tried to remove the influence of the interrelationships of o, p and s upon the coefficient r_{ps}.

(5) All the coefficients measuring the relationship between number of pods and number of seeds are lowered by thus correcting for r_{po} and r_{os}, that is $_{o}r_{ps} < r_{ps}$, always. Several of the partial correlations have the negative sign. Their mean value while very small, $_{o}r_{ps} = + .051$, is apparently significantly positive.

(6) Thus on the average, there is some correlation between the numbers of pods per plant and the number of ovules which develop into seeds which is in part at least independent of—although it may be inseparably bound up with—the morphogenetic factors which link together the magnitudes of the two characters p and o. This correlation which must have its origin in the factors underlying the fertilization of the ovule and its nutrition during the period of growth into a seed, I have ventured to designate as more truly physiological, although there is probably in reality no sharp line of demarcation between physiological and morphogenetic in problems of the kind under consideration here.

CARNEGIE INSTITUTION OF WASHINGTON.

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THE EFFECTS OF ACID AND ALKALINE SOLUTIONS UPON THE WATER RELATION AND THE METABOLISM OF PLANTS[1]

Alfred Dachnowski

The problem of the physiological water requirement of plants (4) is essentially only a phase of that greater problem,—the quantity of water retained by living organisms, by cells and tissues, under a variety of normal and pathological conditions, or during development and the evolution of succulency (of fruits etc.) in the higher plants. It is clearly evident that an attempt to answer this question should also be a step toward an analysis of the ways and means by which tissues and cells hold their normal or abnormal amount of water, *i. e.*, the forces which are active in the process of absorption and retention of water.

Investigators of late years have sought the explanation of the variations in the amount of water absorbed and retained by plants, as well as by animals, in differences in osmotic pressure, and more recently a theory has been proposed to account for it on the basis of the variable "affinity" of colloids for water. The pages that follow concern themselves with a consideration of a few experiments and with the inquiry whether the acceptance of the suggestion here advanced merely necessitates a revision of explanations or whether it adds another to the forces already considered as active in the water relation of plants.

A review in detail of the arguments which have been brought for and against the osmotic conception of water absorption by cells (8), or the one from the point of view of the state of colloids (7,17), seems out of place at this time, since these are questions which on the basis of the facts now available can not be decided as yet. Though probably overrated, the two theories have contributed the experimental data upon which depends much of the fundamental progress of the physicochemical physiology of organisms.

The experiments detailed below have been made with the view toward establishing experimentally what importance, if any, hydrolytic reactions may have in determining the amount of water absorbed and retained by plants during germination and growth. That the velocity and the equilibrium point of hydrolysis may be altered by acids and alkalies is suggested by a number of facts (1), but the conception that such changes may control the course of metabolism and the physiological water requirement of plants needs to be placed on a firmer basis. Attention has been given both to a series of experiments with seeds and with cuttings of plants.

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[1] Contribution from the Laboratory of Plant Physiology, Ohio State University.

Dry seeds of *Phaseolus multiflorus* and *Zea mais* were used which had been in the laboratory for at least three years and proved to be of a low germinating power. The seeds were weighed and placed in glass-covered crystallizing dishes, each containing 100 cc. of solution. At various intervals, the seeds were removed from the solution, carefully dried with filter paper and weighed. The difference in weight, *i. e.*, in the amount of water retained, with the gain or loss on the part of each set of seeds was calculated also in percentage of the original weight of the dry seeds.[2] The data contained in tables I to VI indicate the course of water retention as observed in the seeds. The results show the relative differences in the increase until a maximal point is reached, after which the retention of water lessens. An increase in the amount

TABLE I

THE WATER CONTENT OF BEAN SEEDS (*Phaseolus multiflorus*) IN ACID SOLUTIONS

Two seeds in each 100 c.c. solution

Time Interval in Hours		H_2O	H_2SO_4 *n*/800	HNO_3 *n*/800	HCl *n*/800	HCl *n*/3,200	HCl *n*/6,400
Hours	Minutes						
		2.090	1.995	2.055	2.580	2.240	2.580
2		2.692	2.400	2.080	2.835	2.261	2.720
6	30	3.508	2.862	2.120	3.510	2.519	3.120
16	30	4.220	3.758	2.901	4.305	3.960	4.211
20	30	4.220	4.025	3.312	4.545	4.320	4.549
26	30	4.341	4.330	4.018	5.180	4.912	5.160
40	30	4.463	4.501	4.320	5.452	5.258	5.495
48	30	4.397	4.472	4.370	5.478	5.280	5.500
65		4.430	4.497	4.384	5.510	5.260	5.615
89	30	4.365	4.580	4.403	5.520	5.220	5.630
116		4.250	4.440	4.409	5.540	5.155	5.600
137	30	4.230	4.400	4.445	5.465	4.970	5.535
164	30	4.223	4.203	4.468	5.410	4.840	5.360
195		4.220	4.197	4.450	5.418	4.800	5.352
Maximum percentage increase..........		212.9%	228.8%	217.2%	214.7%	235.7%	218.2%

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[2] The final dry weight of the seeds was not determined, and hence the differences in the amount of material acted upon by the solutions are not known in this set of seeds. The data obtained more recently are reserved for a future publication.

of either acid or alkali above these limits retards the reaction, and finally when sufficient acid or alkali is present the catalytic action is completely arrested. There is a constantly increasing diffusion of by-products from the seeds into the solution surrounding them. Accurate measurements of the hydrogen or hydroxyl ion concentration in the reacting mixtures were not attempted in this case. It is clear from these experiments that the variations in the amount of water absorbed and retained must be due to changes which are induced by the solutions within the cells and tissues of the seeds. Briefly stated, the following are the conclusions of importance in this discussion:

1. Seeds of *Phaseolus multiflorus* swell more and retain greater quantities of water in the solution of any acid than in distilled water (table I).

2. The amount of water that seeds absorb and retain in an acid solution is not dependent upon the concentration of the acid, and is not a function of it. A maximum is attained above which a further increase in the concentration of the acid does not lead to a greater retention of water but to a diminishing one. The decrease in weight is due in part to a loss of food constituents of the cells and is consequent upon a series of changes in the cells and tissues through which their physico-chemical state is progressively altered.

3. When equinormal acids are compared the amount of water retained is greater in H_2SO_4 than in HCl or in HNO_3. The two acids first named are about equally dissociated and yield a higher concentration of hydrogen ions than the equinormal HNO_3, but the amount of water retention induced seems to be determined not by the concentration so much as by the effect of the anions of the particular acid concerned. The order of the effectiveness of the anions in accelerating the water content is SO_4, Cl and NO_3.

4. The addition to the solution of HCl $n/800$ of any salt not reacting with the acid does not decrease the quantity of water absorbed and retained by seeds of *Phaseolus* (table II). The amount retained is still further increased if K_2SO_4 is added. However, a higher concentration of any salt is followed by an inhibition in the capacity for absorbing and retaining water. The effects of molecularly equivalent salt solutions in a solution of $n/800$ hydrochloric acid are not only unequal in degree but in their time reaction as well. The rate at which the absorbing and retaining power for water develops and passes away in the seeds is most rapid with K_2SO_4. In the series of

TABLE II

EFFECT OF HCl n/800 SOLUTION WITH VARIOUS EQUIMOLECULAR SALT SOLUTIONS UPON THE WATER CONTENT OF PHASEOLUS SEEDS

Two seeds in each 100 c.c. solution

Time Interval in Hours		H_2O	HCl n/800	50 c.c. HCl n/800 + 50 c.c.							
Hours	Minutes			$CaCl_2$ n/800	NaCl n/800	KCl n/800	KNO_3 n/800	K_2SO_4 n/800	$C_{12}H_{22}O_{11}$ n/800	$C_6H_{12}O_6$ n/800	$C_2H_5NO_2$ n/800
		2.090	2.580	2.450	2.190	2.110	2.520	2.390	2.420	2.540	2.005
2		2.692	2.835	2.480	2.330	2.325	2.640	2.820	2.860	3.430	2.050
6	30	3.508	3.510	2.750	2.770	3.070	3.510	3.521	3.325	4.260	2.280
16	30	4.220	4.305	3.900	4.320	4.750	5.215	4.710	5.035	5.270	3.155
20	30	4.220	4.545	4.192	4.610	4.930	5.450	5.105	5.270	5.360	3.520
26	30	4.341	5.180	4.778	4.851	5.075	5.655	5.700	5.400	5.500	4.180
40	30	4.463	5.452	5.330	4.932	5.105	5.780	5.860	5.350	5.570	4.830
48	30	4.397	5.478	5.415	4.960	5.100	5.805	5.790	5.330	5.542	4.915
65		4.430	5.510	5.480	4.970	5.070	5.835	5.640	5.260	5.480	4.930
89	30	4.365	5.520	5.000	4.801	4.750	5.850	5.305	4.862	5.435	4.770
116		4.250	5.540	4.850	4.740	4.740	5.520	5.210	4.941	5.425	4.620
137	30	4.230	5.465	4.850	4.725	4.720	5.480	5.190	4.980	5.421	4.581
164	30	4.223	5.410	4.750	4.660	4.680	5.450	5.200	5.010	5.178	4.580
195		4.220	5.418	4.790	4.690	4.690	5.440	5.070	5.040	5.470	4.572
Maximum percentage increase............		212.9%	214.7%	224.5%	226.8%	241.9%	232.1%	245.1%	212.2%	219.2%	246.5%

salts having a common anion the order of effectiveness of the kation is K, Na and Ca, the ion most effective in bringing about an increase in the retention of water being placed first. The order of the effectiveness of the anions is SO_4, Cl and NO_3. This order is nearly identical with that in which the different acids affect the water retaining capacity of seeds.

5. The addition of equimolecular solutions of non-electrolytes to an *n*/800 HCl solution (table II) does not increase to any extent the amount of water retained by seeds. Sucrose brings about greater depression through its presence than dextrose. The most striking exception to the lack of antagonistic action of non-electrolytes is glycocoll. The amino acid accelerates, apparently, both hydrolytic and certain synthetic reactions. An instance of the catalytic action of amino acids is referred to by Dakin (6).

6. The conclusions for the results on the absorption and retention of water by seeds in the alkaline solutions are the analogue of those for the acids (table III). Seeds of *Phaseolus multiflorus* absorb and retain more water in the solution of any alkali than in distilled water. Only within certain limits of concentration is there an increase in the

TABLE III

THE WATER CONTENT OF BEAN SEEDS (*Phaseolus multiflorus*) IN EQUINORMAL ALKALINE SOLUTIONS

Two seeds in each 100 c.c. solution

Time Interval in Hours		H_2O	KOH *n*/800	NH_4OH *n*/800	$Ca(OH)_2$ *n*/800	NaOH *n*/800
Hours	Minutes					
		2.090	2.890	2.020	2.051	2.260
2		2.692	2.930	2.550	2.110	2.910
6	30	3.508	3.081	3.650	2.240	3.240
16	30	4.220	3.992	4.255	3.220	3.405
20	30	4.220	4.477	4.300	3.861	3.560
26	30	4.341	5.350	4.380	4.239	4.195
40	30	4.463	6.650	4.480	4.500	4.930
48	30	4.397	6.745	4.500	4.563	4.980
65		4.430	6.725	4.545	4.622	5.060
89	30	4.365	6.650	4.532	4.660	4.950
116		4.250	6.323	4.428	4.690	4.922
137	30	4.230	6.407	4.315	4.700	4.843
164	30	4.223	6.410	4.220	4.692	4.845
195		4.220	6.460	4.151	4.688	4.810
Maximum percentage increase..........		212.9%	233.4%	225.%	224.4%	223.8%

quantity of water retained, and after this point is exceeded a further increase or decrease in concentration is followed by a diminution in the amount of water held. When equinormal solutions are compared, the amount of water absorbed and retained by seeds is greater in some alkalies than in others. Seeds of *Phaseolus* swell more in KOH than in NH_4OH, and more in either of these than in CaOH or NaOH in the order named. The kations Ca and Na are apparently more active in bringing about a reduction in the water content of cells than either NH_4 or K,—an order of effectiveness nearly the same as in the results with the molecularly equivalent salt solutions in a solution of HCl.

7. Upon comparison of the amounts of water absorbed and retained in equinormal solutions of acids and alkalies (tables I and III) it is found that seeds of *Phaseolus* swell less and retain much less water in an acid medium than in an alkaline solution.

The course of the absorption and retention of water in corn seeds (*Zea mais*) corroborates many of the various results stated above (tables IV to VI). The seeds show in particular a greater water content in solutions of acids and alkalies than in distilled water; they retain varying amounts of water in equinormal solutions of

TABLE IV

THE WATER CONTENT OF CORN SEEDS (*Zea mais*) IN ACID SOLUTIONS

Four seeds in each 100 c.c. solution

Time Interval in Hours		H_2O	H_2SO_4 *n*/800	HNO_3 *n*/800	HCl *n*/800	HCl *n*/3,200	HCl *n*/6,400
Hours	Minutes						
		1.100	1.050	1.070	1.180	1.050	1.035
2		1.270	1.230	1.300	1.390	1.310	1.211
6	30	1.400	1.340	1.390	1.500	1.390	1.319
16	30	1.540	1.460	1.510	1.620	1.480	1.435
20	30	1.563	1.500	1.540	1.670	1.510	1.460
26	30	1.642	1.565	1.600	1.735	1.550	1.510
40	30	1.715	1.630	1.680	1.805	1.580	1.580
48	30	1.750	1.660	1.700	1.830	1.615	1.610
65		1.800	1.702	1.725	1.880	1.620	1.642
89	30	1.802	1.702	1.750	1.880	1.640	1.675
116		1.800	1.720	1.735	1.905	1.640	1.690
137	30	1.800	1.725	1.726	1.921	1.625	1.690
164	30	1.800	1.750	1.704	1.950	1.630	1.660
195		1.800	1.720	1.700	1.939	1.630	1.658
Maximum percentage increase..........		163.6%	166.6%	164.5%	165.2%	156.2%	163.2%

different acids and alkalies; there is the analogous lack of relationship between concentration and the capacity for water retention: an optimal point is reached beyond which a further concentration of the acid or alkali is not followed by a greater water content but by a lesser one; the effectiveness of the anion SO_4 of the particular acid concerned is the same; the increase is greater in alkaline than in equally concentrated acid solutions; the order of the effectiveness of the anions of equimolecular salt solutions in an acid medium (table V) is the same as that observed in the series above; and the action of the non-electrolytes is comparatively similar in this regard.

Important differences, however, exist between the two kinds of seeds which are not without their physiological interest. The amount of water that may be taken up by corn seeds is much smaller than that which can be retained by the seeds of the bush bean. The results for the anions of the equinormal acid and alkaline solutions are more nearly alike than those for the kations. The general grouping of K and Ca for seeds of *Zea mais* (table VI) is the reverse of that observed in *Phaseolus multiflorus*, Ca being more active in bringing about an increase in the water content than K, and this more than Na. The velocity of the reaction is decreased when sodium takes the place of calcium. As the increase in weight between Ca and K in these experiments is considerable, the difference in the reaction must be looked for not in the external conditions of temperature, etc., for they were the same, but in the essential differences of the cell constituents of the two kinds of seeds and their reactions in these solutions.

Another point of interest in this connection is the definite and distinct decrease in the capacity of corn seeds to absorb and retain water with any addition of osmotically equivalent concentrations of certain salts (table V). The anions Cl and NO_3 are again nearly alike in the degree of their effectiveness in lowering the water content, while the kations Ca and K increase the amount of water retained by the seeds. Sucrose shares with the electrolytes Na and K the marked power of reducing the water retaining capacity of seeds in an acid solution; on the other hand, dextrose and glycocoll produce a definite acceleration, but the former more so than the latter when starches are affected.

These results seem to justify the general conclusion that the variations in the water content of seeds cannot be brought about solely through the concentration of acids or alkalies within the cells and

TABLE V

EFFECT OF HCl *n*/800 SOLUTIONS WITH VARIOUS EQUIMOLECULAR SALT SOLUTIONS UPON THE WATER CONTENT OF *Zea mais* SEEDS

Four seeds in each 100 c.c. solution

Time Interval in Hours		H_2O	HCl *n*/800	50 c.c. HCl *n*/800 + 50 c.c.							
Hours	Minutes			$CaCl_2$ *n*/800	NaCl *n*/800	KCl *n*/800	KNO_3 *n*/800	K_2SO_4 *n*/800	$C_{12}H_{22}O_{11}$ *n*/800	$C_6H_{10}O_6$ *n*/800	$C_2H_5NO_2$ *n*/800
		1.100	1.180	1.100	1.060	1.130	1.090	1.060	1.120	1.150	1.120
2		1.270	1.390	1.290	1.272	1.380	1.300	1.280	1.330	1.380	1.372
6	30	1.400	1.500	1.385	1.370	1.483	1.380	1.370	1.431	1.485	1.460
16	30	1.540	1.620	1.500	1.480	1.610	1.480	1.500	1.562	1.610	1.580
20	30	1.563	1.670	1.530	1.500	1.630	1.520	1.525	1.590	1.655	1.622
26	30	1.642	1.735	1.610	1.563	1.690	1.585	1.580	1.650	1.735	1.681
40	30	1.715	1.805	1.680	1.600	1.750	1.660	1.630	1.700	1.805	1.755
48	30	1.750	1.830	1.710	1.621	1.750	1.680	1.660	1.720	1.820	1.760
65		1.800	1.880	1.765	1.650	1.780	1.720	1.700	1.772	1.845	1.810
89	30	1.802	1.880	1.850	1.660	1.800	1.733	1.730	1.800	1.890	1.840
116		1.800	1.905	1.875	1.655	1.815	1.740	1.750	1.800	1.880	1.865
137	30	1.800	1.921	1.875	1.670	1.825	1.740	1.765	1.800	1.900	1.870
164	30	1.800	1.950	1.855	1.690	1.810	1.750	1.795	1.800	1.970	1.880
195		1.800	1.939	1.853	1.685	1.818	1.750	1.820	1.800	1.920	1.872
Maximum percentage increase.		163.6%	165.2%	170.4%	159.4%	161.5%	160.5%	171.6%	160.7%	171.3%	167.8%

tissues. The alterations produced by the variety of substances here used are more easily understood on the hypothesis that hydrolytic cleavages are taking place whereby the water component in the seeds varies the greater in proportion the nearer the equilibrium point reaches to the position of complete hydrolysis. Neither the osmotic pressure of the cell contents is raised to any considerable extent,—for the solutions in which the seeds are kept show increasingly larger amounts of diffusing products of the reaction which in the case of the hydroxides alter the catalyst itself,—nor can any conception of colloidal swelling alone be brought into harmony with the maximal values of water retained, or with the series of chemical changes actually taking place, through which the seeds are progressively altered.

TABLE VI

THE WATER CONTENT OF CORN SEEDS (*Zea mais*) IN EQUINORMAL ALKALINE SOLUTIONS

Four seeds in each 100 c.c. solution

Time Interval in Hours		H_2O	KOH *n*/800	NH_4OH *n*/800	$Ca(OH)_2$ *n*/800	NaOH *n*/800
Hours	Minutes					
		1.100	1.130	1.080	1.090	1.010
2		1.270	1.430	1.325	1.315	1.250
6	30	1.400	1.510	1.430	1.400	1.340
16	30	1.540	1.632	1.566	1.525	1.430
20	30	1.563	1.658	1.604	1.560	1.460
26	30	1.642	1.710	1.640	1.620	1.520
40	30	1.715	1.770	1.720	1.711	1.583
48	30	1.750	1.803	1.750	1.726	1.608
65		1.800	1.832	1.812	1.771	1.666
89	30	1.802	1.855	1.860	1.825	1.685
116		1.800	1.870	1.877	1.863	1.680
137	30	1.800	1.830	1.890	1.860	1.710
164	30	1.800	1.830	1.840	1.855	1.710
195		1.800	1.830	1.832	1.845	1.709
Maximum percentage increase...........		163.6%	171.7%	175.%	176.4%	169.3%

There are now at our disposal a few data which may be utilized in the further consideration of the problem of the relation of transpiration to the water content of growing plants. The plants used in the experiments discussed below were tomato cuttings of known green weight and as nearly alike as possible in transpiration surface. The method pursued has been described in previous publications (3). The plants were fastened to perforated stoppers by means of small amounts of cotton and transferred to sterilized glass bottles of 250 c.c. capacity. Each solution contained one plant and each experiment was continued for 15 to 20 days under greenhouse conditions. A record was made every five days of the weight of water absorbed, the quantity transpired, and the gain or loss in the weight of plants. The curves of figures 1 to 4 are based upon the data contained in tables IX to X, and indicate graphically the course of the water relation. It may be remarked, incidentally, that the physiological reactions of the plants proceed at an unequal pace in the various solutions, even though the external conditions which affect the rate are kept alike. The noteworthy points are that the progress, as represented graphically, attains in almost all cases a maximum on the fifth day; thence the rate falls off rapidly,—with a recoil and nearly proportional lowering on the tenth day; after the adjustment has occurred there is a gradual increase in responsiveness to the solution which becomes more characteristic on the fifteenth day. A moment's study of the data or of a few of the curves will show how hazardous are conclusions concerning the stimulating or inhibiting effect of a solution when based upon results made at arbitrary intervals of time or under unlike conditions. Another point of interest is the fact that during the first five days, stimulation and the rate of the reaction is much more rapidly effected by acid than by the same concentration of alkali (tables VII and VIII). A very striking contrast is obtained also by observing that an increase in size of the root system is not necessarily connected with an accelerating action upon absorption or transpiration (tables IX to XII).

The chief results of the experiments may be described as follows:

1. During a period of fifteen days, tomato cuttings absorb and transpire less water in an acid solution of the concentration here employed than in distilled water (table VII). An exception is H_2SO_4 $n/3200$. The plants absorb more water in a HNO_3 $n/800$ than in an equinormal HCl solution, and less in sulfuric and acetic acids in the order named. There is a great difference in the relationship between the quantity absorbed and transpired and the concentration of the acid. A point is reached in the solution of HCl and H_2SO_4 beyond which a further increase or decrease in concentration is followed by a diminished absorption and loss of water, while in solutions of HNO_3 and CH_3COOH the absorption and transpiration of water varies inversely as the concentration.

2. At the concentrations employed the absorption and transpira-

TABLE VII

WATER RELATION OF TOMATO CUTTINGS IN ACID SOLUTIONS

Values in grams for 15 days

Solution	Absorbed			Transpired			Retained			Remarks
	5th Day	10th Day	15th Day	5th Day	10th Day	15th Day	5th Day	10th Day	15th Day	
1. H_2O	8.790	5.920	8.600	8.085	5.855	8.390	0.705	0.065	0.210	Roots 2–5 mm.
2. HCl *n*/800	6.866	1.830	2.120	6.590	1.880	3.010	0.276	−0.050	−0.890	Plant wilted; immersed portion of stem gelatinized; no roots.
3. HCl *n*/3,200	11.720	5.340	5.780	10.965	5.230	5.660	0.755	0.110	0.120	Roots 1–2 mm.
4. HCl *n*/6,400	9.080	5.010	5.570	8.545	4.945	5.450	0.535	0.065	0.120	Roots 2–5 mm.
5. H_2SO_4 *n*/800	8.350	1.080	0.020	8.230	1.260	0.710	0.120	−0.180	−0.690	Plant wilted; immersed portion of stem gelatinized; no roots.
6. H_2SO_4 *n*/3,200	16.100	6.750	1.440	15.260	6.675	1.335	0.840	0.075	0.105	Roots 1–3 mm.
7. H_2SO_4 *n*/6,400	10.965	4.060	3.710	10.325	4.125	3.850	0.640	−0.065	−0.140	Roots 2–4 mm.
8. HNO_3 *n*/800	12.205	2.030	0.102	11.940	2.000	1.552	0.265	0.030	−1.450	Plant dead. Immersed portion of stem gelatinized; no roots.
9. HNO_3 *n*/3,200	11.820	5.090	2.410	11.180	5.070	2.405	0.640	0.020	0.005	Roots 2–3 mm.
10. HNO_3 *n*/6,400	14.105	4.230	3.505	13.230	4.260	3.360	0.875	−0.030	0.145	Roots 2–5 mm.
11. CH_3COOH *n*/800	5.390	0.960	0.360	6.100	1.080	1.470	−0.710	−0.120	−1.110	Plant dead. Immersed portion of stem gelatinized.
12. CH_3COOH *n*/3,200	7.515	1.380	1.170	7.940	1.310	1.310	−0.425	0.070	−0.140	Plant wilting; no roots.
13. CH_3COOH *n*/6,400	11.050	4.390	4.550	10.330	4.860	4.500	0.720	−0.470	0.050	Roots 2–9 mm.

Atmometer 49 c.c.–33 c.c.–28 c.c.
Temperature 8°–35° C.
Rel. humidity 38%–100%.
Barometer 29.15–29.95 cm.

tion of water by tomato plants in alkaline solutions is less than in distilled water (table VIII). An exception is the KOH *n*/6400 solution. The alkalies show the following order of effectiveness in which the kation bringing about the least inhibition is placed first: K, Na, Ca, NH_4. As in the case of the acids, there is no relationship between increase in concentration of alkali and the increase in the amount of water absorbed and transpired. Beyond a certain optimal point a further increase or decrease in concentration leads to a diminished water relation.

3. If the quantities of water absorbed and transpired in equinormal solutions of acids and alkalies are compared, the serial weighings show that tomato plants function better in an alkaline medium than in one of acid.

In tables VII and VIII are seen also the effects of the solutions upon the amounts of water retained by the plants. In every case the acid inducing the higher absorption and transpiration increased the retention of water. This correspondence is not so marked, however, in the case of the alkaline solutions 5, 7, 12, and 13 (table VIII).

The wilting observed in solutions 2, 5, 8, 11, and 12 (table VII) is the result of transpiration exceeding absorption, while that in solutions 7, 10, and 13 (table VII) and 4 (table VIII) as well as that in 3, 4, 5, 6, 7, 12 and 13 (table IX), 4 (table X) is primarily a loss in food constituents within the plant. Similar facts were mentioned in an earlier paper (5). These few data upon wilting in relation to the different solutions show nearly as many wilting coefficients for plants as there are solutions, atmospheric conditions and energy relations within the plants.

The reduction of the water content to incipient wilting and permanent wilting can be studied more easily in this manner, and the method here used should be of great use in ecological investigations on that account.

4. The addition to a solution of HCl *n*/800 of any salt in the same molecular concentration inhibits in all cases the action of the acid and increases the amount of water absorbed and transpired. The different salts are unequally effective in this regard. The curves in figures 1 to 4 represent graphically the course of the reaction. In the series of chlorides (table IX) K is more powerful in producing an increase in the water requirement of the plants than Na or Ca. The general grouping of these ions is very similar to that given for the kations in

table VIII on the effects of different alkalies. In the series of sulfates and nitrates, Na is more effective than K or Ca. A comparison of the effectiveness of the anions needs no further comment. The order is NO_3, SO_4 and Cl, very nearly that of the grouping of anions in the acid solution (table VII). The order of the anions, as that of the kations, is not always readily apparent as an inspection of the serial weighings will show. While in the last five or ten days of the experiment the order is as given above, it may appear inversely in the earlier part of the experiment. The changes are undoubtedly due to the removal of food constituents and to alterations in the contents of cells.

The marked effect of glycocoll among the non-electrolytes in counteracting the action of hydrochloric acid is not shared by dextrose or sucrose. The latter has the least effect of any of the various salts added upon the quantity of water absorbed and transpired by tomato plants.

5. The presence of any salt in equimolecular concentration in the solution of an alkali does not in all cases increase the amount of water absorbed and transpired by tomato plants. In table X the difference in the quantities of the water relation induced through the action of electrolytes and non-electrolytes of the same concentration, it is easily noted, is not as great as in an acid solution.

The effect of any salt seems to be made up of the sum of the effects of the constituent ions. The kations arrange themselves in about the following order in which the most effective in increasing the water supply is placed first in the series: Ca, Na, K. The relation in the order of the anions is not readily apparent, but it is of interest to note that in an alkaline solution the nitrate of calcium brings about a greater reduction in the available water for transpiration and absorption than the nitrate of potassium.

If the amounts are compared which the plants absorb and transpire in acid and alkaline solutions through the action of equimolecular solutions of salts added to them, it will be seen that the plants function more evenly in an alkaline medium.

Non-electrolytes, through their presence in osmotically equivalent concentrations, reduce the water relation of tomato plants in an alkaline medium, but not to the extent as in an acid solution. Comparison readily shows that the amount of the difference is considerably above that in distilled water or in HCl *n*/800.

TABLE VIII

WATER RELATION OF TOMATO CUTTINGS IN ALKALINE SOLUTIONS

Values in grams for 15 days

Solution	Absorbed			Transpired			Retained			Remarks
	5th Day	10th Day	15th Day	5th Day	10th Day	15th Day	5th Day	10th Day	15th Day	
1. H_2O	8.790	5.920	8.600	8.085	5.855	8.390	0.705	0.065	0.210	Roots 2–5 mm.
2. KOH *n*/800	6.910	7.390	8.840	6.560	7.280	8.530	0.350	0.210	0.310	Roots 3–12 mm.
3. KOH *n*/3,200	4.225	3.900	6.310	3.990	3.770	6.100	0.235	0.130	0.210	Roots 8–20 mm.
4. KOH *n*/6,400	15.240	7.980	13.780	14.500	8.760	12.550	0.740	−0.780	1.230	Roots 3–16 mm.
5. NH_4OH *n*/800	4.835	4.870	5.470	4.450	4.760	5.210	0.385	0.110	0.260	Roots 2–3 mm.
6. NH_4OH *n*/3,200	5.550	5.580	8.390	5.070	5.400	8.190	0.480	0.180	0.200	Roots 3–8 mm.
7. NH_4OH *n*/6,400	4.430	3.770	6.790	4.065	3.660	6.520	0.365	0.110	0.270	Roots 3–5 mm.
8. $Ca(OH)_2$ *n*/800	5.670	7.640	8.660	5.300	7.390	8.370	0.370	0.250	0.290	Roots 10–55 mm.
9. $Ca(OH)_2$ *n*/3,200	3.460	6.415	9.790	3.130	6.250	9.500	0.330	0.165	0.290	Roots 10–22 mm.
10. $Ca(OH)_2$ *n*/6,400	7.310	4.690	7.070	7.010	4.620	6.800	0.300	0.070	0.270	Roots 3–12 mm.
11. NaOH *n*/800	4.460	7.060	10.630	4.015	6.840	10.270	0.445	0.220	0.360	Roots 10–23 mm.
12. NaOH *n*/3,200	2.870	5.015	4.525	2.530	4.880	4.420	0.340	0.135	0.105	Roots 3–5 mm.
13. NaOH *n*/6,400	7.840	4.635	6.215	7.110	4.580	6.120	0.370	0.055	0.095	Roots 3–5 mm.

Atmospheric conditions as in table VII.

TABLE IX

WATER RELATION OF TOMATO CUTTINGS IN HCl *n*/800 WITH VARIOUS EQUIMOLECULAR SALT SOLUTIONS

Values in grams for 20 days in 5-day periods

Solution	Quantity of Water			Remarks
	Absorbed	Transpired	Retained	
1. H_2O	5.350	4.960	0.390	
	4.120	3.790	0.330	Roots 12–15 mm.
	9.520	9.260	0.260	
	13.260	12.670	0.590	
2. HCl *n*/800	5.040	5.150	−0.110	Plant wilting; immersed portion of stem gelatinized; no roots.
	1.170	1.030	0.140	
	0.830	1.010	−0.180	
	1.560	2.290	−0.730	
3. HCl *n*/800+	12.050	11.525	0.525	Immersed portion of stem partly gelatinized; few short roots 2–3 mm.
	4.480	4.305	0.175	
KCl *n*/800.	4.150	4.130	0.020	
	3.420	3.620	−0.200	
4. HCl *n*/800+	7.750	7.250	0.500	Immersed portion of stem partly gelatinized; roots 2–4 mm.
	2.150	1.930	0.220	
NaCl *n*/800	4.860	4.990	−0.130	
	6.890	6.910	−0.020	
5. HCl *n*/800+	8.330	7.900	0.430	Same as No. 4.
	2.960	2.550	0.410	
$CaCl_2$ *n*/800	4.440	4.580	−0.140	
	4.920	4.920	0.000	
6. HCl *n*/800+	9.810	9.410	0.400	Immersed portion of stem partly gelatinized; roots 1–2 mm.
	4.220	3.970	0.250	
K_2SO_4 *n*/800	4.820	4.840	−0.020	
	5.090	5.140	−0.050	
7. HCl *n*/800+	13.610	12.935	0.675	Immersed portion of stem brownish; few short roots 1–3 mm.
	4.030	3.805	0.225	
Na_2SO_4 *n*/800	4.780	4.790	−0.010	
	5.110	5.170	−0.060	
8. HCl *n*/800+	9.650	9.320	0.330	Lower portion of immersed stem brownish; few short roots 2–5 mm.
	2.660	2.445	0.215	
$CaSO_4$ *n*/800	6.210	6.070	0.140	
	10.180	9.990	0.190	
9. HCl *n*/800+	9.470	9.050	0.420	As above; few short roots 1–4 mm.
	3.790	3.460	0.330	
KNO_3 *n*/800	6.515	6.320	0.195	
	9.755	9.360	0.395	
10. HCl *n*/800+	19.900	19.150	0.750	Immersed portion of stem partly gelatinized; roots 1–2.5 mm.
	6.840	6.540	0.300	
$NaNO_3$ *n*/800	8.580	8.220	0.360	
	9.755	9.360	0.395	

TABLE IX—*Continued*

Solution	Quantity of Water			Remarks
	Absorbed	Transpired	Retained	
11. HCl *n*/800+	9.190	8.810	0.380	As above in No. 10; roots 1–3 mm.
	4.770	4.460	0.310	
$CaNO_3$ *n*/800	8.600	8.310	0.290	
	12.710	12.270	0.440	
12. HCl *n*/800+	9.230	8.920	0.310	Immersed portion of stem gelatinized; roots 1–2 mm.
	2.070	1.980	0.090	
$C_{12}H_{22}O_{11}$ *n*/800	2.250	2.370	−0.120	
	1.460	1.680	−0.220	
13. HCl *n*/800+	8.630	8.300	0.330	Immersed portion of stem gelatinized; brownish; few short roots 1–3 mm.
	3.350	3.190	0.160	
$C_6H_{12}O_6$ *n*/800	4.390	4.190	0.200	
	5.160	5.280	−0.120	
14. HCl *n*/800+	13.800	13.280	0.520	As above in No. 13; roots 1–6 mm.
	5.380	5.110	0.270	
$C_2H_5NO_2$ *n*/800	8.830	8.450	0.380	
	12.470	12.080	0.390	

6. The effects of the reaction of solutions on the quantity of water retained by the plants and on the green weight of roots and tops are most markedly shown in tables XI and XII. In every case where salts have been added to HCl *n*/800, the amount of water retained and the actual gain in the weight of the plants is greater than that in the corresponding hydrochloric acid solution without salts. Especially is this the case where glycocoll and the nitrates of Na, Ca and K had been used. The chlorides are more effective than the sulfates in bringing about a reduction in the amount of water that the plants can retain. The figures also show that the least increase is found in the sucrose solution.

The table (XI), moreover, brings out some interesting data on the changes in the metabolism of the plants. The difference between the actual gain of the plants—*i. e.*, the increase in weight above the initial green weight—and the total amount of water retained, is greater in the nitrates of Na and Ca than in their sulfates or chlorides.

This order of the effectiveness of the anions, it will be noted, is reversed with the salts of K; the acceleration is in the order Cl, SO_4, NO_3. There is no difficulty in discovering that equimolecular concentrations of glycocoll and sodium nitrate when added to HCl *n*/800 are nearly alike in their efficiency to increase the constructive processes in general, and that NaCl and sucrose induce the greatest depression in the developmental reactions. In the one case both the release of energy and the accumulation of food material are stimulated in the presence of these salts, while in the other case metabolic reactions and the process of translocation of food constituents are considerably retarded. No relationship to transpiration is apparent. Where the selective action of the salts favors metabolism and growth and the leaf surface increases, transpiration is consequently greater also. The relationship, however, is only approximately so. The absorption of water and the absorption of salts are not identical processes (4).

7. In table XII are shown in a similar way the comparative effects of the presence of equimolecular concentrations of salts in an alkaline solution. In almost all cases, the amount of water retained and the actual gain in the green weight of plants is larger than that in distilled water. But when compared with the corresponding solution of KOH *n*/800 lacking any one of these salts the great difference in the amounts induced through the action of electrolytes and non-electrolytes is readily noted. It is apparent that at the same concentration of alkali the sulfate of calcium is more effective in increasing the water content of the plants than the sulfate of sodium. The order in which the anions are grouping themselves is not as readily made out as in the case of the acid solutions. The causes for these changes, here as in several of the examples cited above, are undoubtedly several in kind, and largely due to the chemical changes taking place within the cells and tissues of the plants. There is no difficulty in recognizing the following order in which the kations are effective, that one producing the greatest reaction being given first: Ca, Na; the position of K is somewhat variable. Again no relationship to the amount of water transpired is noticeable; the selective activity of the salts and their beneficial or injurious effect is independent of the solute. This lack of relationship is most marked upon comparison with the non-electrolytes, and upon observing the reaction of the various salt solutions on the metabolism of the plants. The greatest difference between the quantity of water retained and the actual gain in the green weight of the plants is obtained in $CaCl_2$; the least difference is found in KCl. Of extreme importance in this connection is the gain in the weight of plants over and above the amount of water retained by them in the alkaline solutions of glycocoll and sucrose. There seems scarcely any doubt that the organic compounds accelerate both the hydrolytic and the synthetic reactions.

TABLE X

WATER RELATION OF TOMATO CUTTINGS IN KOH *n*/800 WITH VARIOUS EQUIMOLECULAR SALT SOLUTIONS

Values in grams for 20 days in 5-day periods

Solution	Quantity of Water			Remarks
	Absorbed	Transpired	Retained	
1. H_2O	5.350	4.960	0.390	Roots 12–15 mm.
	4.120	3.790	0.330	
	9.520	9.260	0.260	
	13.260	12.670	0.590	
2. KOH *n*/800	12.660	12.290	0.370	Roots 15–30 mm.
	5.980	5.515	0.465	
	15.170	14.440	0.730	
	22.320	21.595	0.725	
3. KOH *n*/800+ KCl *n*/800	6.420	6.200	0.220	Roots 5–11.5 mm.
	3.410	3.020	0.390	
	8.075	7.870	0.205	
	14.405	13.875	0.530	
4. KOH *n*/800+ NaCl *n*/800	10.980	9.615	1.365	Roots 20–50 mm.
	6.670	7.025	−0.355	
	20.185	19.165	1.020	
	28.875	28.195	0.680	
5. KOH *n*/800+ $CaCl_2$ *n*/800	12.720	13.270	0.450	Roots 20–35 mm.
	6.810	6.250	0.560	
	19.690	18.535	1.155	
	28.480	27.685	0.795	
6. KOH *n*/800+ K_2SO_4 *n*/800	7.640	7.350	0.290	Roots 20–40 mm.
	4.470	4.050	0.420	
	12.490	12.090	0.400	
	20.090	19.560	0.530	
7. KOH *n*/800+ Na_2SO_4 *n*/800	5.555	5.065	0.490	Roots 15–30 mm.
	4.125	3.975	0.150	
	11.250	10.820	0.430	
	19.100	18.620	0.480	
8. KOH *n*/800+ $CaSO_4$ *n*/800	11.560	11.110	0.450	Roots 20–65 mm.
	8.990	8.030	0.960	
	25.870	24.745	1.125	
	33.760	32.690	1.070	
9. KOH *n*/800+ KNO_3 *n*/800	9.390	8.930	0.460	Roots 20–25 mm.
	4.870	4.420	0.450	
	11.300	10.720	0.580	
	19.410	18.675	0.735	
10. KOH *n*/800+ $NaNO_3$ *n*/800	8.810	8.465	0.345	Roots 20–35 mm.
	4.895	4.425	0.470	
	15.475	14.765	0.710	
	29.450	28.850	0.600	

TABLE X—*Continued*

Solution	Quantity of Water			Remarks
	Absorbed	Transpired	Retained	
11. KOH *n*/800 +	5.000	4.760	0.240	
	4.010	2.850	1.160	Roots 20–40 mm.
$CaNO_3$ *n*/800	20.180	19.660	0.520	
	36.980	36.000	0.980	
12. KOH *n*/800+	5.840	5.280	0.560	Roots 20–40 mm.
	4.780	4.030	0.750	
$C_{12}H_{22}O_{11}$ *n*/800	8.990	8.800	0.190	
	17.660	16.930	0.730	
13. KOH *n*/800+	4.080	3.830	0.250	
	6.940	6.170	0.770	Roots 20–55 mm.
$C_6H_{12}O_6$ *n*/800	16.390	15.770	0.620	
	26.440	25.750	0.690	
14. KOH *n*/800+	6.630	6.380	0.250	
	5.760	5.120	0.640	Roots 20–80 mm.
$C_2H_5NO_2$ *n*/800	18.330	17.485	0.845	
	31.640	30.575	1.065	

A comparison regarding the qualitative as well as the quantitative differences between the effects of acids and those of alkalies indicates that in an alkaline medium the metabolic processes and the translocation of food materials go on more readily than under the influence of an acid medium and that the presence of a salt in the one solution gives rise to reactions unlike that in the other medium.

Tentatively the following summary is here presented, the ecological and agricultural significance of which will be evident from the discussion of the results:

1. Different acids and alkalies are unequal in their effectiveness to modify the water relation of plants. At one concentration the influence may be related to the chemical characteristics of the solute concerned, at another concentration it may involve the physicochemical properties of the solution. The velocity of the action is independent of the concentration of the solution; it increases to a maximum and with further concentration not only inhibits biochemical processes but destroys them. As to the physiological effects of the solutions the order in which acids and alkalies induce absorption and transpiration of water is often different from the order in which the water content or the metabolism of the plants is altered.

2. The effect of adding equimolecular salt solutions of different

kinds to an acid or an alkaline solution shows that the reactions are specific and unlike with each salt employed (pages 421, 423). The order of effectiveness in inhibiting or in producing an increase in the water relations of plants may be stated as follows:

(*a*) In a solution of hydrochloric acid the salts of sodium counteract to a marked degree the injurious effects of the acid and are preferable to potassium salts. The nitrates of any of these three bases give uniformly better results than the sulfates or chlorides.

Calcium has a greater accelerating action in an unbalanced acid solution if combined with a sulfate, while potassium proves most corrective if used as a chloride. The beneficial effects obtained may be due to an indirect reaction between the different substances in the medium through changes induced by the plant. The acid radicle (NO_3) is usually absorbed more rapidly, leaving behind the base ion or alkaline radicle (Na), which then may give rise to a direct reaction between the different substances in the medium.

(*b*) In an alkaline medium (KOH) calcium salts are more beneficial and should be furnished preferably in the form of a sulfate. Sodium salts are more capable of altering the water relation of plants as chlorides, and potassium salts give uniformly a greater increase as nitrates. The relative effects of the anions and kations may be accounted for in a manner as pointed out in (*a*).

3. The remarkable increase in weight which has attended the use of non-electrolytes, especially glycocoll and sucrose, proves ready digestibility of these organic compounds, their relative value in the maintenance and repair of constituents destroyed during growth in acid and alkaline solutions, and further indicates that water retention during growth may be limited by any factor which prevents the construction of food constituents, *i. e.* the chemosynthesis of proteins and carbohydrates. If this interpretation is correct, it follows that artificial organic fertilizers may supplement advantageously the use of mineral salts.

4. The injurious properties of acid solutions (and probably also of acid soils and subsoils) may not necessarily be due to their acid character. In itself acidity is not always a disturbing factor to growth and transpiration of plants. The apparent inhibiting action may be the effect of the presence of some salt, perhaps in large measure the reaction of the solutions after the plants have been growing in them for some time, retarding the rate of hydrolysis of substances in the cells of plants.

Table XI

Transpiration and Growth of Tomato Cuttings in HCl *n*/800 with Various Equimolecular Salt Solutions

Values in grams for 20 days

Solution	Quantity of Water			Gain or Loss in Weight of Plants	Extent of Chemical Changes within Tissues	Weight of Roots
	Absorbed	Transpired	Retained			
1. H_2O	32.250	30.680	1.570	1.140	−0.430	0.185
2. HCl *n*/800	8.600	9.480	−0.880	−1.040	−0.160	0.000
3. HCl *n*/800 + KCl *n*/800	24.100	23.580	0.520	0.190	−0.330	0.010
4. HCl *n*/800 + NaCl *n*/800	21.650	21.080	0.570	0.450	−0.120	0.014
5. HCl *n*/800 + $CaCl_2$ *n*/800	20.650	19.950	0.700	0.490	−0.210	0.018
6. HCl *n*/800 + K_2SO_4 *n*/800	23.940	23.360	0.580	0.315	−0.265	0.019
7. HCl *n*/800 + Na_2SO_4 *n*/800	27.530	26.700	0.830	0.500	−0.320	0.010
8. HCl *n*/800 + $CaSO_4$ *n*/800	28.700	27.825	0.875	0.630	−0.245	0.020
9. HCl *n*/800 + KNO_3 *n*/800	28.530	28.290	1.340	1.105	−0.235	0.020
10. HCl *n*/800 + $NaNO_3$ *n*/800	45.075	43.270	1.805	1.350	−0.455	0.018
11. HCl *n*/800 + $CaNO_3$ *n*/800	35.270	33.850	1.420	1.100	−0.320	0.028
12. HCl *n*/800 + $C_{12}H_{22}O_{11}$ *n*/800	15.010	14.950	0.060	−0.305	−0.245	0.010
13. HCl *n*/800 + $C_6H_{12}O_6$ *n*/800	21.530	20.960	0.570	0.360	−0.210	0.015
14. HCl *n*/800 + $C_2H_5NO_2$ *n*/800	40.480	38.820	1.660	1.345	−0.315	0.200

5. A further fact of interest from a study of the physiological reactions of the different solutions toward plants here employed and growing under the same group of external conditions is the observation that conceptions of colloidal swelling or a differential osmotic pressure in cells and tissues are in themselves inadequate to account for the varying amounts of water retained by plants, and can not be considered the fundamental factors in absorption. The values of the quantity of water retained do not increase progressively with every increase in the concentration of acid or alkali in the solution surrounding the plants. This leads to the conclusion that the influence of acids and alkalies may be accounted for on the basis of their hydrolyzing power upon the various colloidal and other constituents within the cells. The diffusion of food constituents of seeds out of the cells into the solution surrounding them does not permit an interpretation of the variability in their water content on the basis of differences in osmotic pressure, or from the point of view of that series of physicochemical phenomena designated as colloidal. Both are unquestionably involved and of importance in the general problem of the water content of plants, but they may well represent only a phase of that greater series of phenomena which is included under the term of hydrolytic reactions.

It is fairly well known that the final equilibrium is not the same in the case of enzyme action as with acids or bases. During the catalytic reactions a number of intermediate compounds arise. The hydrolytic processes are carried farther by acids than by enzymes, and they bring about the greatest changes in materials and in energy transformations, probably owing to diffusion of the products of the reaction. The effects of alkalies are variable, due in large measure to the alteration of the catalyst itself by the reaction, *i. e.*, through the combined effect of neutralization and the production of salts with the by-products of the reaction. Hydrolytic and synthetic reactions induce the conditions which favor the retention of water and underlie irregularities in growth as well as the maintenance of a constant weight over long periods of time.

TABLE XII

TRANSPIRATION AND GROWTH OF TOMATO CUTTINGS IN KOH *n*/800 WITH VARIOUS EQUIMOLECULAR SALT SOLUTIONS

Values in grams for 20 days

Solution	Quantity of Water			Gain or Loss in Weight of Plants	Extent of Chemical Changes within Tissues	Weight of Roots
	Absorbed	Transpired	Retained			
1. H_2O	32.250	30.680	1.570	1.140	−0.430	0.185
2. KOH *n*/800	56.130	53.840	2.290	1.950	−0.340	0.410
3. KOH *n*/800 + KCl *n*/800	32.310	30.965	1.345	1.220	−0.125	0.160
4. KOH *n*/800 + NaCl *n*/800	66.710	64.000	2.710	2.520	−0.190	0.480
5. KOH *n*/800 + $CaCl_2$ *n*/800	67.700	64.740	2.960	2.175	−0.785	0.440
6. KOH *n*/800 + K_2SO_4 *n*/800	44.690	33.050	1.640	1.420	−0.220	0.310
7. KOH *n*/800 + Na_2SO_4 *n*/800	40.030	38.480	1.550	1.390	−0.260	0.250
8. KOH *n*/800 + $CaSO_4$ *n*/800	80.180	76.575	3.595	3.265	−0.330	0.830
9. KOH *n*/800 + KNO_3 *n*/800	44.970	42.745	2.225	1.750	−0.475	0.260
10. KOH *n*/800 + $NaNO_3$ *n*/800	58.630	56.505	2.125	1.850	−0.275	0.300
11. KOH *n*/800 + $CaNO_3$ *n*/800	66.170	63.270	2.900	2.640	−0.280	0.460
12. KOH *n*/800 + $C_{12}H_{22}O_{11}$ *n*/800	37.270	25.040	1.230	1.815	+0.585	0.530
13. KOH *n*/800 + $C_6H_{12}O_6$ *n*/800	53.850	51.520	2.330	2.030	−0.300	0.510
14. KOH *n*/800 + $C_2H_5NO_2$ *n*/800	62.360	59.560	1.800	2.775	+0.975	0.700

Water not retained in this manner is usually allowed to escape as transpirational water loss. The simple fact that the quantity absorbed is equivalent under certain conditions to the amount transpired does not indicate that the mere consumption of water leads to growth. The growth of plants and their distribution depends in large part upon the amount of water retained within the plants, but the degree of the water-holding capacity (*e. g.*, xerophytism in plants and the succulence of certain species) is not a function of mere water consumption, *i. e.*, of the rate of supply of water to loss by transpiration; nor can it be determined solely from a morphological examination of the structure of the shoot of plants. Transpiration is not primarily the cause of growth. The two are frequently associated and may at times lie so closely together that they give the impression of running parallel with each other in a causal relation. In such cases transpirational values are the consequence of growth *e. g.* in leaf surface. The processes of the absorption of water and that of dissolved substances are not identical but independent of each other.

Another line of evidence in proof of the conception of the chemical changes induced through acids and alkalies and thus reducing or increasing the quantity of retained water, is apparent from a comparison of the differences in the loss of tissue substances or the increase of it by plants under these conditions. The degree of the conversion of colloidal and other cell constituents is paralleled by a change in their affinity for water which is retained from any available source.

The water content of the plants is essentially dependent not only upon the catalytic action of an optimal concentration of acid or alkali but also upon the chemical character of the body material affected. The changes that occur in the cells and tissues may be still further retarded or hastened through the addition of salts.

The results of the experiments described in the foregoing pages show that the reactions obtained from the addition of salts to toxic acid or alkaline solutions may aid in developing further the conception of antagonistic relations among salts. It is not proposed to discuss the views which have been suggested from time to time in regard to the causes of this phenomenon or the manner in which salts correct injurious effects. The effects may be due in part to depression of ionization (11), or to the formation of undissociated salts (2). The valence of ions (12) or their lowered rate of absorption (19) and adsorption (16) may determine the action, and it may be referred to complicated changes in the permeability of the plasmatic membrane of the cells of plants (13, 15), or to the effect of the salts upon protein compounds (9, 10, 20, 21, 14). The results here reported make it probable, and the conclusion seems unavoidable, that no one of the various hypotheses advanced seems to consider the quantitative changes taking place in the material and the energy system within the cells, whereby the contents become altered in their water retaining capacity through catalytic reactions which accelerate or retard the rate of the hydrolysis of substances in the cells.

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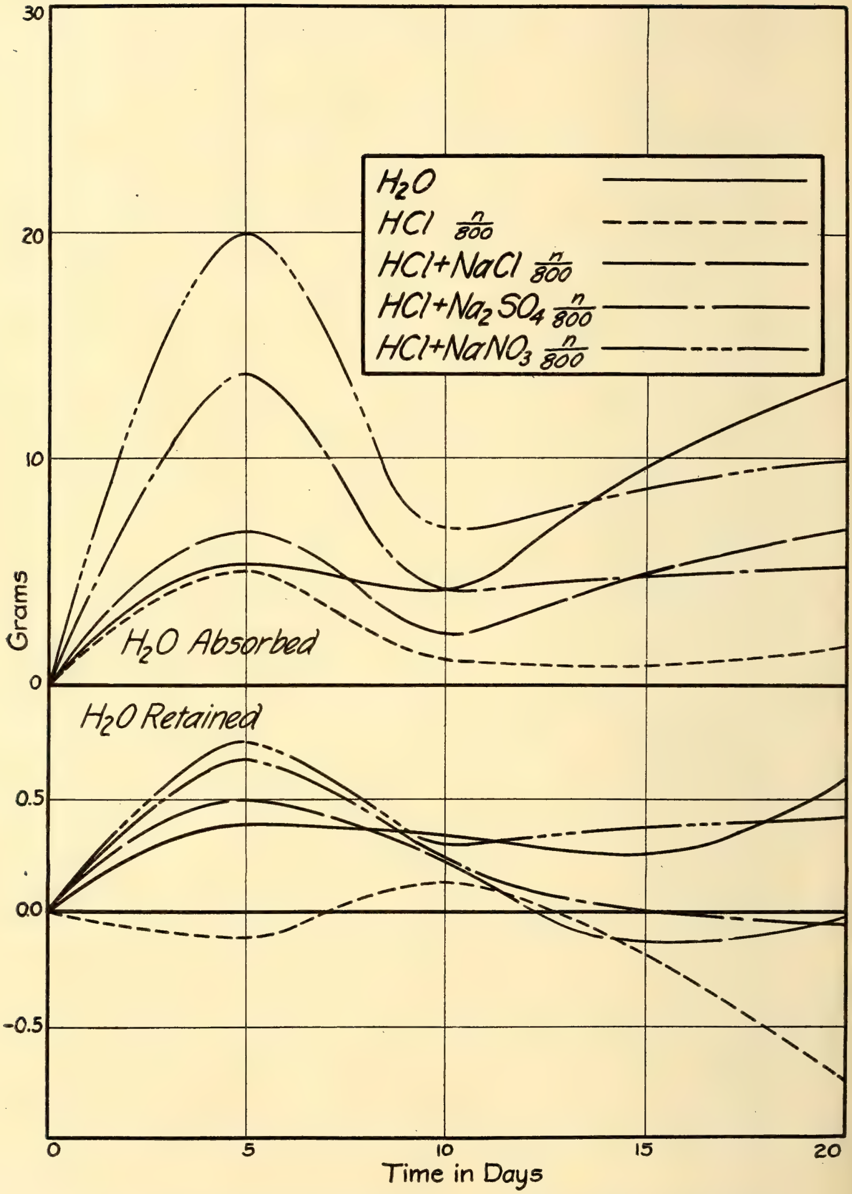

FIG. 1. The effectiveness of anions in a toxic acid solution, (table IX).

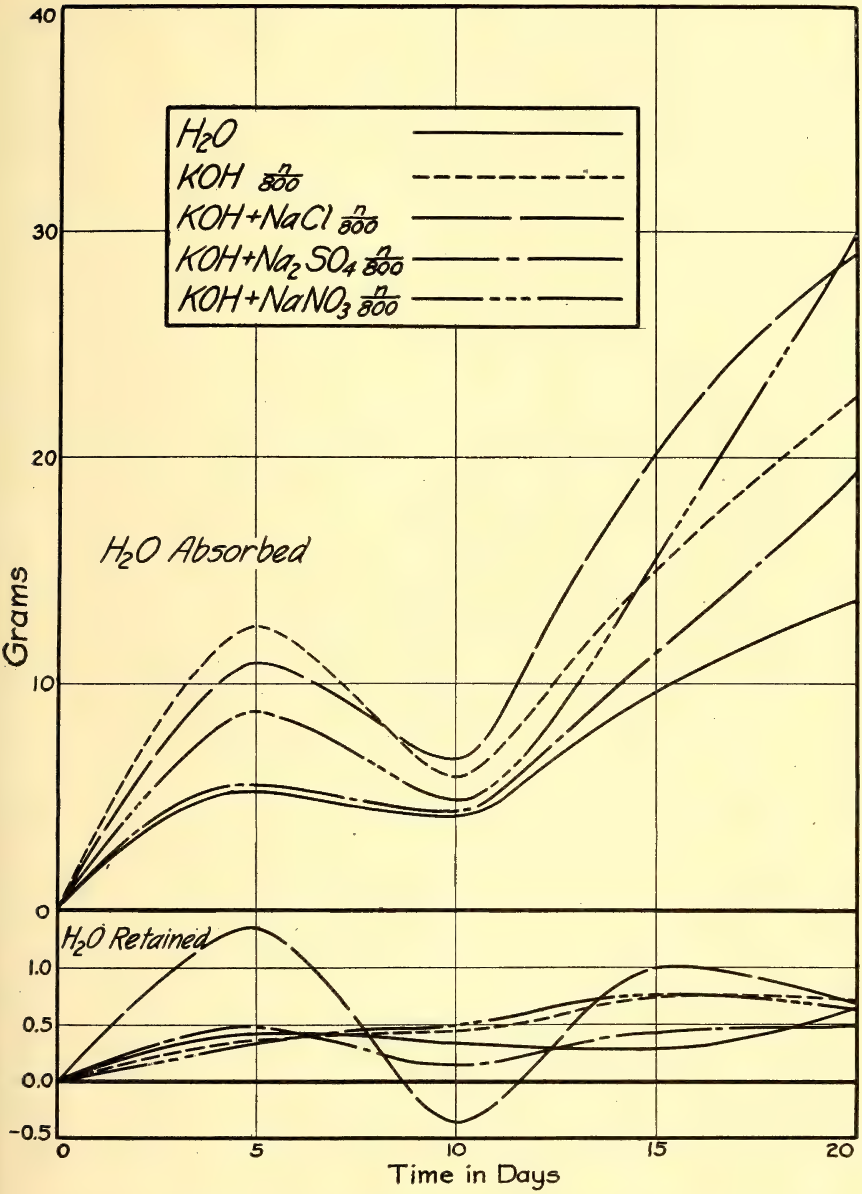

FIG. 2. The effectiveness of anions in a toxic alkaline solution, (table X).

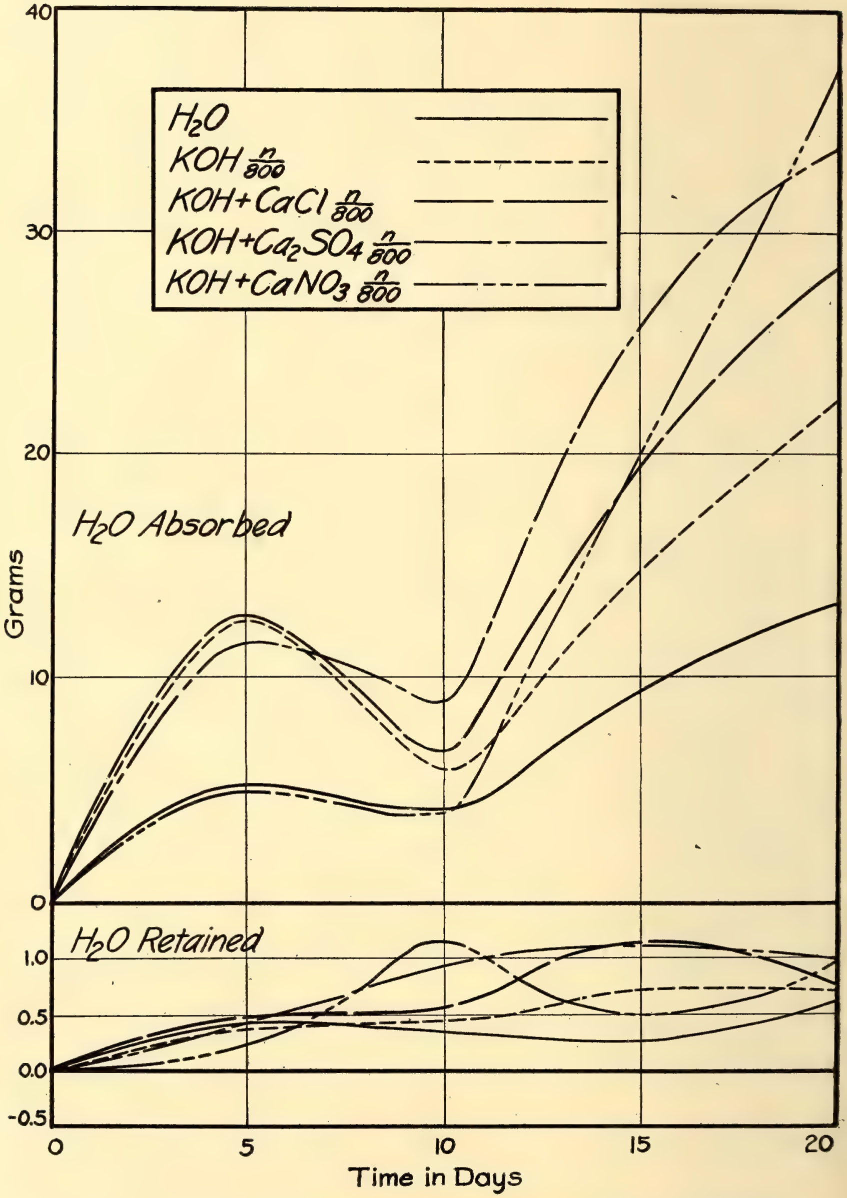

FIG. 3. The effectiveness of anions in a toxic alkaline solution, (table X).

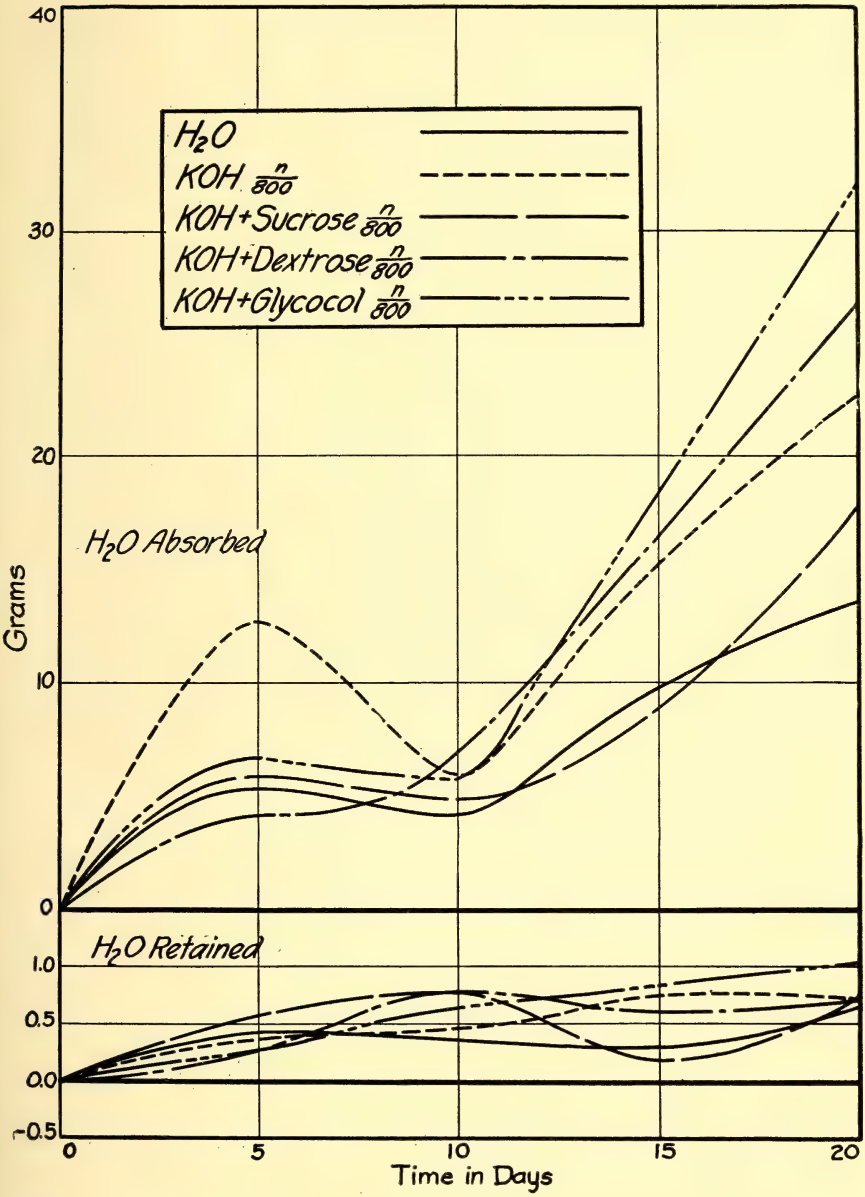

FIG. 4. The effectiveness of non-electrolytes in a toxic alkaline solution, (table X).

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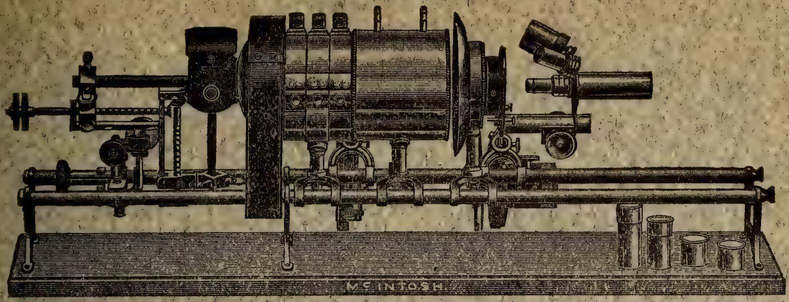

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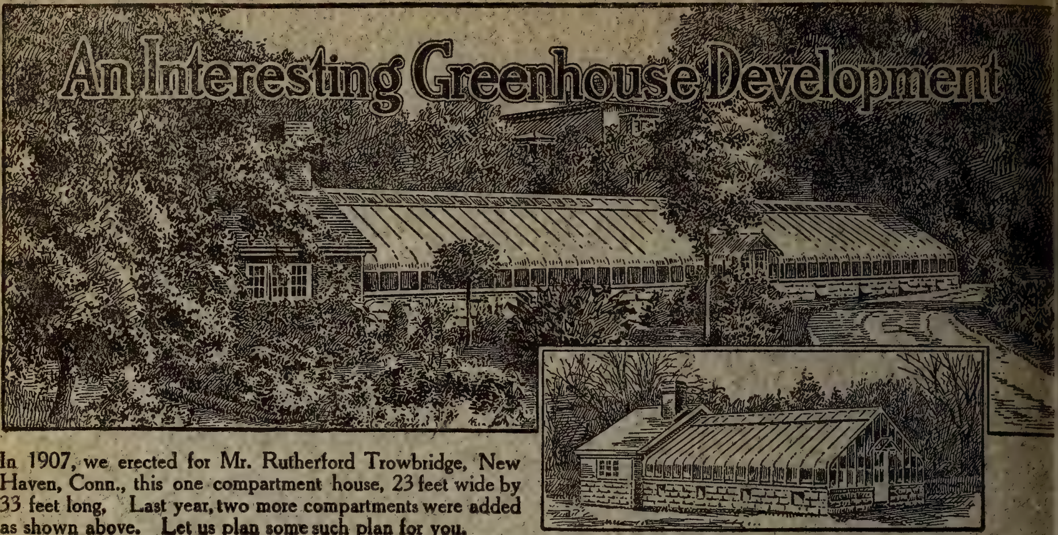

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INVESTIGATIONS ON THE PHYLOGENY OF THE ANGIOSPERMS

3. Nodal Anatomy and the Morphology of Stipules

Edmund W. Sinnott and Irving W. Bailey

The morphology of stipules has been a much debated subject since careful study of plant structures began. These generally small and inconspicuous appendages at the base of the petiole, which occur so constantly in some families of angiosperms and which are so invariably absent in others, have been regarded variously as "accessory leaves," as independent organs, as a "product of the leaf base of the primordial leaf," as the remains of the two lateral leaflets of a three-compound leaf, as an incomplete axillary ligule, as a reduced leaf-sheath or as the remains of such a sheath of fused leaves as occurs in Equisetum. Almost everyone who has written on the subject has had a different idea as to just what the nature of stipules is and what has been the cause of their origin; and the relations between stipules, leaf-sheath, ligule, tendril, petiolar spine and ochrea have been widely discussed by many botanists. The following paper has as its object a presentation of certain anatomical facts, apparently overlooked hitherto, which seem to be of importance in connection with this general problem.

One of the writers (3) has recently made a comparative study of the anatomy of the node throughout the angiosperms and has come to certain conclusions as to the evolutionary development of the various types of nodal structure in that group. The facts and conclusions in his paper may be briefly summarized as follows.

[The **Journal** for October (1: 357–440) was issued 31 Oct. 1914.]

1. In vascular plants below the Gnetales and angiosperms the foliar trace, whether it is a single bundle or is composed of two or of many strands, causes at its departure only a single break or gap (if any) in the continuity of the woody ring of the stem.

2. Among angiosperms the nodal topography is much more various for there may be a single gap (unilacunar type), three distinct and usually distant gaps (trilacunar type), or many gaps (multilacunar type). The nodal plan is exceedingly constant within large groups, most families and many orders being almost invariably characterized by some one of the three types.

3. From its predominance in the lower Archichlamydeae and especially in the presumably primitive Amentiferae, Ranales and Rosales, and from its occurrence in the more primitive members of otherwise unilacunar or multilacunar families or orders, the trilacunar type is regarded as the most ancient angiosperm condition.

4. The unilacunar node has evidently been derived by a reduction of the trilacunar, either through the approximation of the three gaps and their coalescence into one; or through the disappearance of the two lateral bundles and gaps.

5. The multilacunar type has been derived by an amplification of the three original bundles and gaps into five, seven, nine or more.

6. The multilacunar node of the monocotyledons has apparently been derived from such a trilacunar condition as persists in the Potamogetonaceae and in the seedlings of other families.

The importance of these facts of nodal anatomy in connection with the morphology of stipules, sheathing leaf-bases and related structures is evident when we observe that *in the majority of plants with a trilacunar node, stipules are present; that in almost all with a unilacunar node, stipules are absent, and that in all with a multilacunar node, the leaf has a more or less sheathing base.* There is obviously an intimate connection between the type of nodal topography and the occurrence of stipules.

The following table[1] indicates briefly the facts as to the occurrence of stipules and related structures in the more important families of dicotyledons, together with the prevailing type of nodal topography (and and also of leaf margin) in each.

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[1] Parentheses indicate an uncommon condition. The three types of nodal topography are represented by the figures 1, 3 and ∞. The prevailing character of the leaf margin in each family is noted in cases where both types are not well represented.

Family	Stipules	Node	Margin
Casuarinaceae	0	1	Entire
Piperaceae	+ or 0	3 or ∞	Entire
Chloranthaceae	+	3 or ∞	Toothed
Salicaceae	+	3	Toothed
Garryaceae	0	3	Entire
Myricaceae	+ or 0	3 or 1	Both
Leitneriaceae	0	3	Entire
Juglandaceae	0	3(5)	Both
Julianaceae	0	3	Entire
Betulaceae	+	3	Toothed
Fagaceae	+	3	Toothed
Ulmaceae	+	3	Toothed
Urticaceae	+	3	Toothed
Moraceae	+ or Sheath	3 or ∞	Entire
Proteaceae	0	3	Toothed
Santalaceae	0	1	Entire
Olacaceae	0	3	Entire
Loranthaceae	0	1	Entire
Aristolochiaceae	0	3	Entire
Polygonaceae	Sheath	∞	Entire
Chenopodiaceae	0	1	Entire
Amarantaceae	0	1	Entire
Nyctaginaceae	0	1	Entire
Phytolaccaceae	0	1	Entire
Aizoaceae	0, (+)	1	Entire
Portulacaceae	+	1	Entire
Caryophyllaceae	0, (+)	1	Entire
Trochodendraceae	+ or 0	1, 3, 5	Toothed
Ranunculaceae	+ or Sheath	3 or ∞	Toothed
Lardizabalaceae	0	3	Entire
Berberidaceae	+	3 or ∞	Toothed
Menispermaceae	0	3	Entire
Magnoliaceae	+, 0, or Sheath	3, 1, or ∞	Entire
Calycanthaceae	0	3	Entire
Anonaceae	0	1	Entire
Myristicaceae	0	1	Entire
Monimiaceae	0	1	Both
Lauraceae	0	1	Entire
Hernandiaceae	0	1	Entire
Papaveraceae	0, (+) or Sheath	3 or 1	Toothed
Capparidaceae	+ or 0	1	Entire
Cruciferae	0	3 or 1	Both
Resedaceae	Minute	1	Both
Crassulaceae	0	3 or 1	Entire
Saxifragaceae	+ or 0	3, (5)	Toothed
Pittosporaceae	0	3	Entire
Cunoniaceae	+	3	Toothed
Hamamelidaceae	+	3	Both
Platanaceae	+	7	Toothed
Crossosomataceae	0	3	Entire
Rosaceae	+, (0)	3, (1, 5)	Toothed
Connaraceae	0	3	Entire
Leguminosae	+	3, (5)	Entire

Family	Stipules	Node	Margin
Geraniaceae	+ or 0	3	Toothed
Oxalidaceae	0, (+)	3	Entire
Tropaeolaceae	0, (+)	3	Both
Linaceae	+ or 0	3	Both
Zygophyllaceae	+	3	Entire
Rutaceae	0, (+)	3, (1)	Entire
Simarubaceae	0	7	Entire
Burseraceae	0, (+)	5	Entire
Meliaceae	0	5	Entire
Malpighiaceae	+	3, (1)	Entire
Vochysiaceae	0, (+)	1	Entire
Tremandraceae	0	1	Entire
Polygalaceae	0	1	Entire
Euphorbiaceae	+ or 0	3, (1)	Both
Buxaceae	0	1	Entire
Empetraceae	0	1	Entire
Coriariaceae	0	1	Entire
Anacardiaceae	0, (+)	3	Both
Cyrillaceae	0	1	Entire
Aquifoliaceae	0, (+)	3, (1)	Both
Celastraceae	Minute or 0	1	Toothed
Staphylleaceae	+	3	Both
Aceraceae	0	3	Toothed
Hippocastanaceae	0	3, (5)	Toothed
Sapindaceae	+ or 0	3	Both
Balsaminaceae	0	1	Toothed
Rhamnaceae	+	3	Both
Vitaceae	+	3, 5, 7	Toothed
Elaeocarpaceae	+	3	Both
Tiliaceae	+	3	Toothed
Malvaceae	+	3, (∞)	Toothed
Sterculiaceae	+	3	Both
Dilleniaceae	+ or 0	3 or 1	Both
Eucryphiaceae	+	3	Toothed
Ochnaceae	+	3	Toothed
Marcgraviaceae	0	1	Entire
Theaceae	0	1	Both
Guttiferae	0, (+)	1	Entire
Dipterocarpaceae	+	3 or 5	Entire
Cistaceae	+ or 0	1	Entire
Bixaceae	+	3	Toothed
Violaceae	+	3	Toothed
Flacourtiaceae	+	3	Both
Stachyuraceae	+	3	Toothed
Passifloraceae	+	3	Both
Caricaceae	0	3 or ∞	Toothed
Begoniaceae	+	3	Toothed
Peneaceae	Minute	1	Entire
Oliniaceae	0	1	Entire
Thymeleaceae	0	1	Entire
Eleagnaceae	0	1	Entire
Lythraceae	Minute or 0	1	Entire
Punicaceae	0	1	Entire

Family	Stipules	Node	Margin
Lecythidaceae	0	1	Entire
Rhizophoraceae	+	3	Entire
Nyssaceae	0	3	Entire
Alangiacae	0	3	Entire
Combretaceae	0	1	Entire
Myrtaceae	0	1	Entire
Melastomataceae	0	1	Entire
Oenotheraceae	0, (+)	1	Both
Araliaceae	Sheath	∞	Both
Umbelliferae	Sheath	∞	Toothed
Cornaceae	0	3	Entire
Clethraceae	0	1	Toothed
Pirolaceae	0	1	Both
Ericaceae	0	1	Entire
Epacridaceae	Sheath	∞	Entire
Diapensiaceae	0	1	Both
Theophrastaceae	0	1	Both
Myrsinaceae	0	1	Entire
Primulaceae	0	1	Both
Plumbaginaceae	+ or Sheath	3	Entire
Sapotaceae	0, (+)	1	Entire
Ebenaceae	0	1	Entire
Symplocaceae	0	1	Toothed
Styracaceae	0	1	Entire
Oleaceae	0	1	Entire
Loganiaceae	+ or 0	1	Both
Gentianaceae	0 or Sheath	1 or ∞	Entire
Apocynaceae	0, (+)	1	Entire
Asclepiadaceae	0	1	Entire
Convolvulaceae	0, (+)	1	Entire
Polemoniaceae	0	1	Entire
Hydrophyllaceae	0	1	Entire
Borraginaceae	0	1	Entire
Verbenaceae	0	1	Both
Labiatae	0	1	Toothed
Solanaceae	0	1	Both
Scrophulariaceae	0	1	Both
Bignoniaceae	0	1	Entire
Gesneraceae	0	1, (3)	Both
Acanthaceae	0	1	Entire
Myoporaceae	0	1	Entire
Plantaginaceae	0	3	Both
Rubiaceae	+	1, (3)	Entire
Caprifoliaceae	+ or 0	3, (5)	Both
Valerianaceae	0	3	Both
Dipsacaceae	0	3	Toothed
Cucurbitaceae	0	3	Toothed
Campanulaceae	0	1	Both
Goodeniaceae	0	3 or 5	Both
Compositae	+ or 0	3 or ∞	Both

A perusal of this table makes plain the following facts. Of the 75 families which possess stipules or leaf sheaths, 53 are characteristically tri- or multilacunar and in 5 others this type of node is very common. 16 of these stipulate families are characteristically unilacunar, but in 11 of them stipules are either very rare or minute, and in the others (Portulacaceae, Capparidaceae, Cistaceae, Loganiaceae and Rubiaceae) they are often poorly developed or absent. Of the 81 families, on the other hand, which are totally without stipules 52 are characteristically unilacunar and 2 more are frequently so; and 27 have three or more traces. Of the 78 typically tri- or multilacunar families, 53 have stipules. Of the 70 typically unilacunar ones, 57 are without stipules and in the other 13 these structures are usually rare or inconspicuous. The families in which the leaf base is characteristically sheathing, such as the Polygonaceae, Araliaceae and Umbelliferae, practically always have a multilacunar node. It is thus apparent that stipules and lateral leaf traces generally occur together. The exceptions to this rule will be discussed later.

The following particular cases are of interest in showing the relation between nodal anatomy and the presence of stipules.

The Polygonaceae are prevailingly multilacunar and in almost every case possess a stipular structure, the ochrea, which completely encircles the stem. The dioecious species of Rumex, however, are exceptional in the family in having a trilacunar node and also in possessing two typical and distinct stipules instead of an ochrea.

The Aquifoliaceae are generally unilacunar and stipules are either absent or very small among them. In *Ilex opaca*, however, which has two lateral traces and gaps, the stipules reach their best development in the family.

The Rosaceae are typically trilacunar and stipulate. Spiraea and its allies, however, are exceptional in being unilacunar and also exstipulate.

Certain genera of the Dilleniaceae are trilacunar and others unilacunar. Stipules are generally absent in the family, but the few cases where they occur are only in the trilacunar genera.

Almost all of the Umbelliferae are multilacunar and have sheathing leaf-bases. Hydrocotyle, however, is exceptional in being trilacunar and in having two typical and distinct stipules.

The intimate connection between stipules and the two lateral bundles of a trilacunar leaf-trace is therefore very apparent. A study of the nodal anatomy suggests an explanation for this fact, for the stipules are invariably inserted directly opposite the points of origin of the two lateral bundles (*figs.* 1, 4 and 5), really as continuations of the two swellings on the surface of the stem caused by the exit of the traces. If we go back to the primordium of such a leaf at the growing point we find that it usually begins as a three-lobed structure, the central lobe giving rise to the petiole and blade and the lateral ones to the stipules. These three lobes mark the future position of the three leaf-traces. In subsequent development the stipules usually grow considerably, often equalling or exceeding the young blade for a time (*fig.* 2), and still showing their relation to the three swellings which mark the position of the procambial strands that are to give rise to the leaf-traces. The traces, however, when they are finally formed, do not enter the three primordial lobes of the leaf but all three converge into the central one, which is to form the petiole and blade. Each stipule obtains its vascular supply, if it has one, from branches derived from the corresponding lateral traces (*figs.* 1 and 5). These facts as to the topography of the vascular system at the base of the petiole and as to the innervation of the stipules have been worked out by numerous investigators, particularly by Colomb (1). He makes the origin of the vascular supply to the stipules their most distinguishing character and defines a stipule as "an appendage inserted on the stem at the base of the leaf, all the bundles of which are derived entirely from the corresponding foliar bundles."

The significant fact, however, seems to be the intimate connection between the stipules and the swellings opposite the lateral leaf-traces This is even manifest in the case of stipules which are adnate to the petiole for a considerable distance, as in certain of the Rosaceae and many other plants; for here the base of the free portion of each stipule (its oldest part) is directly opposite the point of origin of one of the lateral traces (*fig.* 3), although the subsequent growth of the basal portion of the leaf (*a–b*) carries the stipules up and renders their connection with the lateral bundles, except in very young leaves, less obvious. In a unilacunar node there is of course only one swelling (*fig.* 6) and this developes directly into the petiole and blade without an accompanying formation of stipules. All these facts suggest the conclusion that the early growth of the tissue which is to form the lateral traces exerts a stimulating influence which results in the formation of a considerable body of tissue, the stipule. It is almost as if a whole leaf began to be developed opposite each departing trace (as in the lower vascular plants) but that only the middle one persisted. There certainly appears to be some sort of morphogenetic connection between lateral leaf-trace and stipule.

In the case of sheathing leaf bases and the polygonaceous ochrea we really have a row of adjacent stipules (each opposite one of the numerous leaf-trace bundles) which have become fused together. Sometimes, especially in the case of five-bundle nodes, there are two broad-based stipules, as in most of the Vitaceae, each opposite a pair of traces. When the bundles and gaps are more numerous and occur round most of the stem periphery, as in the Umbelliferae and many others, the sheath is much broader and its resemblance to two stipules is usually gone; but even in such cases it occasionally manifests itself, as in certain of the Magnoliaceae.

The relation between the stipules of dicotyledons, on the one hand, and the ligule of grasses, the tendril of Smilax and the "stipules" of the Potamogetonaceae, on the other, has been much discussed. A study of the nodal anatomy of these monocotyledonous plants and its relation to the appendages in question is helpful in determining their real nature.

In the more vigorous species of the Potamogetonaceae, a family which modern classifications regard as among the most primitive of monocotyledons, three main bundles enter the base of the leaf just as in the trilacunar dicotyledons, a much simpler condition than that prevailing in most monocotyledons. From the two lateral ones branches are sent off into the stipules (*fig.* 7), so that these organs receive their vascular supply in precisely the same way as do the dicotyledonous stipules. On the basis of this evidence the two structures certainly appear to be homologous. It is significant that both in their nodal anatomy and in the character of their stipular appendages the Potamogetonaceae approach the dicotyledons more closely than do any other monocotyledons.

The two tendrils of Smilax, which are inserted at the base of the petiole, are apparently homologous with the stipules of the Potamogetonaceae, for their vascular supply is largely derived from the lateral members of a trio of large bundles which, with a number of smaller ones, enter the base of the leaf from the axis.

The ligule of grasses, the vascular supply of which was investigated carefully by Colomb, is more complicated anatomically but seems to be homologous with the stipular appendages of the other monocotyledons which we have discussed. Anatomical evidence therefore points toward a connection between nodal topography and stipular appendages in the monocotyledons similar to that which we have noted in the dicotyledons.

Let us now consider the various exceptions to the general rule that lateral leaf-traces and stipules always occur together.

There are about 20 unilacunar families in which stipules are found, but in most of these families they are rare and are almost always thin, scarious or minute. If our theory is correct, these unilacunar families have been derived by reduction from trilacunar (and hence presumably stipulate) ones, and it would therefore be only natural that stipules or vestiges of them should occasionally persist. In many cases the vascular supply of a unilacunar leaf, although causing but a single gap, is composed of three bundles; and the stipules, when present, are often related to the lateral ones of this trio. The interpetiolar stipule of the Rubiaceae is evidently to be regarded as a fusion of two formerly independent and adjacent ones, since each stipule receives its vascular supply from both leaf-traces.

There are also about 30 families which are prevailingly trilacunar but in which stipules are absent or rare. Some of the more important of these are the Juglandaceae, Proteaceae, Menispermaceae, Aristolochiaceae, Lardizabalaceae, Calycanthaceae, Pittosporaceae, Simarubaceae, Burseraceae, Meliaceae, Aceraceae, Plantaginaceae and Cucurbitaceae.[2] Many genera and species in normally stipulate, trilacunar families often lack stipules. The lateral bundles at the node in such plants do not cause the formation of stipules, but directly opposite each lateral trace is often observable a swelling or rounded projection which only needs to be slightly elongated, or to be the seat of a gland or water pore, to become a typical stipule.

If we examine these exstipulate trilacunar families, as set forth in the previous table, we are at once struck by the fact that in the great majority of them (about 75 per cent) the leaves or leaflets are prevailingly *entire*. This suggests the possibility that stipules, on the one hand, and the teeth or lobes of the lamina, on the other, may be dependent for their occurrence on essentially the same factor or factors.

Such an hypothesis is strengthened by a study of the relation between the character of the leaf margin and the occurrence of stipules in those families which have both toothed and entire, and stipulate and exstipulate, leaves. In the Saxifragaceae, for example, the entire-leaved genera Philadelphus, Deutzia and Hydrangea are without stipules, whereas stipules or stipule-like appendages occur in Ribes, which almost always has toothed leaves; and also in certain of the herbaceous serrate genera. In the Caprifoliaceae the entire-leaved genera Lonicera and Diervilla are exstipulate, as are the entire-leaved species of Viburnum, but the serrate, dentate or lobed species of the last-named genus are in the great majority of cases provided with stipules. The Rosaceae are overwhelmingly stipulate, but in the sub-family Chrysobalanoideae, which is mainly tropical and entire-leaved, stipules are either absent or extremely small. The same fact is also evident within single genera, for those species of Salix, for example, which are quite entire, have no stipules or have very small ones; and *Myrica asplenifolia*, with its strongly dentate leaves, is stipulate whereas the other species, many of which are nearly or quite entire, are not provided with stipules. In all these instances the node is trilacunar. In many other families, such as the Euphorbiaceae, Violaceae, Moraceae and others, the absence or small size of stipules in entire-leaved species and their strong development in species with toothed leaves is evident.

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[2] The tendrils of the Cucurbitaceae are perhaps stipular in character.

The resemblance between stipules and leaf-teeth is still further emphasized by the fact that in the young leaf both structures are almost always tipped with water pores or with glands, and that these pores or glands usually become functionless in the mature leaf. Stipules in many cases wither and fall when the leaf comes to maturity. The facts seem to point to a similar functional importance, in the young and growing leaf, of the terminal organs of both stipules and teeth, a function which usually ceases after the leaf has become mature.

The occurrence of stipules therefore seems to be dependent on the structure of both the node and the margin. When the node is trilacunar and the margin toothed, stipules are almost invariably present; and when the node is unilacunar and the margin entire, they are almost invariably absent. The former determining factor is evidently the more important of the two, for the presence of lateral traces seems to be almost essential to the production of stipules, often causing them to be developed in plants the leaves of which are devoid of marginal pores or glands. The presence of such a pore or gland, however, usually seems to be necessary for the production of a definite stipular appendage instead of a mere rounded swelling.

What light, then, do these anatomical facts throw on the vexed question of the morphology of stipules? They lend little support to the theories that these structures are independent organs, vestiges of a sheath of fused leaves or lateral leaflets of a compound leaf, but rather favor the contention that stipules are integral portions of the base of the leaf, a view which is well expressed by Eichler when he says that stipules arise without exception as a product of the leaf-base of the primordial leaf. Anatomical facts are also in agreement with the theory frequently put forward that stipules, the sheathing leaf-base, the ochrea, the stipular appendages of the lower monocotyledons and the tendrils and ligule of the higher ones are morphologically identical, for the character and position of these various structures is to a large extent dependent on the type of nodal topography and the manner in which the base of the leaf is innervated. The so-called stipules of ferns and gymnosperms are not dependent on nodal anatomy and are therefore apparently not to be regarded as homologous with those of angiosperms.

As to just what is the morphological character of stipules we cannot be quite sure, but the results of the present investigation indicate that they may perhaps be considered as the two earliest leaf-teeth, their position being determined by that of the two lateral traces rather than by that of the vascular bundles of the lamina, as in the case of ordinary teeth. They may also be regarded as two basal leaf lobes, although the difference between a tooth and a lobe seems to be more one of degree than of kind. The function of stipules as bud scales in certain families apparently does not indicate their true morphological nature but is rather to be looked upon as a secondary adaptation.

As to the phylogeny of stipules there seems to be considerable doubt. The bulk of opinion, expressed by many writers and recently emphasized by Domin (2) in a series of papers, is that a ligule or sheathing leaf-base is the most primitive condition and that this has gradually degenerated, in many families of dicotyledons, into two stipules. The facts brought forward in the present paper show that the character of the leaf-base, whether sheathing, stipulate or devoid of appendages, depends closely upon whether the node is multilacunar, trilacunar or unilacunar, respectively. The question as to which type of leaf-base is the most ancient is therefore intimately connected with the parallel question as to which type of nodal anatomy is the most ancient. In a previous paper one of the writers has brought forward evidence that the trilacunar node is the most primitive angiosperm condition. If this theory is the correct one a leaf with two independent stipules is evidently more ancient in type than one with a sheathing base, the latter having arisen by an increase in basal extent of the stipules and their gradual fusion with the petiole.

As to whether the earliest angiosperms were provided with stipules or not we may not be sure. If the leaf margins of these plants were devoid of glands or pores, it is very likely that rounded swellings, rather than typical stipules, occurred opposite the lateral leaf-traces, as in the trilacunar entire families today. On the other hand, if primitive angiosperms possessed toothed leaves, it seems altogether probable that they were provided with stipules from the first. The fact that in so many families of plants stipules bear evidences of reduction would rather indicate that they once were a much more conspicuous feature of angiosperm leaves than they generally are at present.

SUMMARY

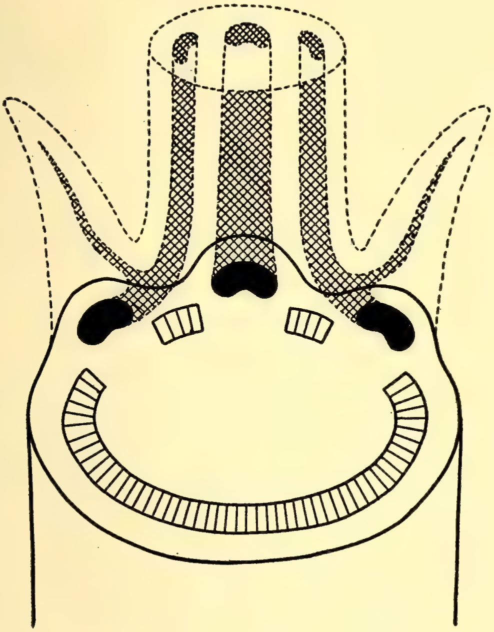
1. There is an intimate connection between the type of nodal anatomy (one, three or many traces and gaps) and the occurrence of stipules and similar structures in dicotyledons. In the majority of plants with a trilacunar node stipules are present; in almost all with a unilacunar node they are absent, and in all with a multilacunar node the leaf has a sheathing base.

2. There is a similar connection in monocotyledons, particularly in such primitive forms as the Potamogetonaceae.

3. The growth of the lateral leaf-trace apparently exerts a stimulus which results in the development of the stipule, for the stipule invariably occurs directly opposite the point of departure of the trace.

4. The character of the leaf margin is also important in governing the occurrence of stipules, for stipules are generally absent in entire-leaves families, even though the latter are trilacunar. The fact that stipules and leaf teeth almost always possess apical pores or glands which are usually atrophied after the leaf has reached maturity suggests that both structures have essentially the same function.

5. Morphologically, stipules are to be regarded as integral portions of the leaf, and seem to be more nearly homologous with teeth than



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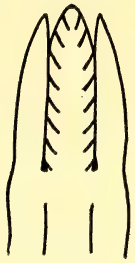

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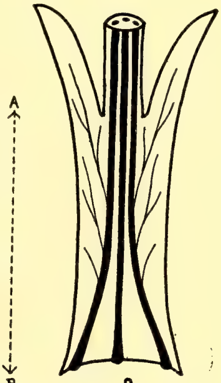

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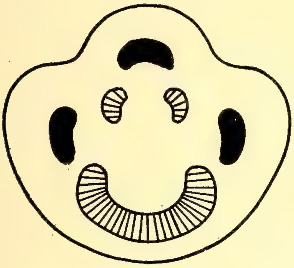

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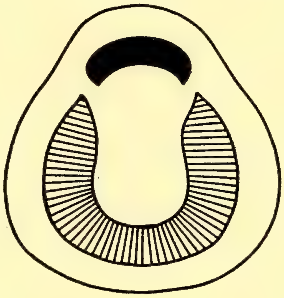

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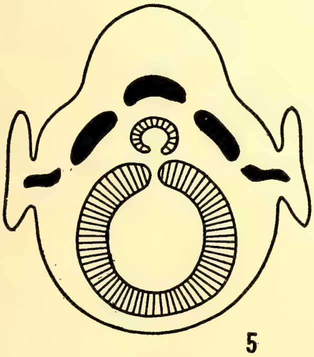

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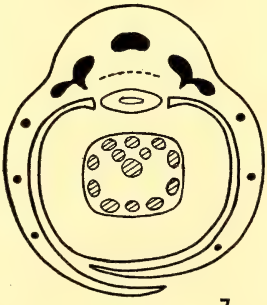

7

SINNOTT AND BAILEY: NODAL ANATOMY.

with any other structures. Stipules, sheaths, ligules and similar modifications of the base of the petiole are dependent in position and character on the anatomy of the node, and seem thus to be essentially homologous.

6. A leaf provided with two distinct stipules is more ancient in type than one with a sheathing base.

BUSSEY INSTITUTION,
HARVARD UNIVERSITY

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DESCRIPTION OF FIGURES OF PLATE XLIV.

FIG. 1. Diagram showing relation of stipules to lateral leaf-traces and course of traces in the base of the petiole.

FIG. 2. Very young leaf of Robinia, showing stipules and blade.

FIG. 3. Base of petiole of Rosa showing relation of free portions of stipules to insertion of lateral leaf-trace bundles.

FIG. 4. Transverse section just below node of Hamamelis (typical trilacunar dicotyledon) showing departure of the three leaf-traces and the swellings opposite them.

FIG. 5. Transverse section a little higher up than in *fig.* 4, showing the formation and innervation of the stipules.

FIG. 6. Transverse section at the node of Eucalyptus (typical unilacunar dicotyledon) showing absence of lateral leaf-traces and stipular swellings.

FIG. 7. Transverse section at node of Potamogeton showing general nodal topography, with formation and innervation of stipules.

OBSERVATIONS ON THE DEVELOPMENT AND GERMINATION OF THE SEED IN CERTAIN POLYGONACEAE

Edward F. Woodcock

The Polygonaceae have been included by Harz (7, p. 1072) under the "Curvembryonaten" which he speaks of as being furnished with an abundant perisperm, and laterally placed embryo. The term "perisperm" is applied to the remaining portion of the nucellus in a mature seed. Harz (p. 1102) carefully figures and describes the buckwheat seed, considering the entire storage region as perisperm, and stating that the same relation holds in species of *Rumex*.

Johnson (11, p. 334), evidently influenced by the work of Harz, was led to believe that there is a rather close relationship between the Polygonaceae and the Piperaceae, in which family he observed perisperm. In these Piperaceae he looks upon the very abundant perisperm as the real storage region, it being separated from the embryo by a layer of endosperm, which, instead of acting as a storage region, serves to digest and pass on food material to the embryo from the perisperm. In an earlier paper (10, p. 368) Johnson had already pointed out this restriction of the endosperm in *Saururus* and suggests the probability of a similar relation existing in all seeds furnished with a large amount of perisperm as in Polygonaceae, Chenopodiaceae, Phytolaccaceae, Caryophyllaceae, etc.

Kraemer (12, p. 262) in his text-book merely mentions the fact that the Polygonaceae possess a "mealy" endosperm, without going into a discussion of the seed development. He also figures in detail a transverse section of *Fagopyrum esculentum*, but states nothing further in regard to its morphology.

Two years later Stevens (18, pp. 59–65) made a detailed study of the morphology of the seed of buckwheat to determine whether, as suggested earlier by Harz and Johnson, perisperm did really exist in the mature seed. After careful investigation he was convinced that no perisperm existed in this seed at maturity. The fact that Humphrey (9), working on the Scitaminales, found, in closely related genera, various amounts of endosperm, suggested to Stevens that even though there is an absence of perisperm in *Fagopyrum esculentum*, the other members of the Polygonaceae may perhaps be characterized by perisperm at maturity. In the order Scitaminales Humphrey found that the Musaceae are characterized by an abundant starch-bearing endosperm, the Zingiberaceae and Cannaceae by a thin layer of aleurone or proteid-containing endosperm, and the Marantaceae by an apparent absence of endosperm in the mature seed. Humphrey also pointed out a variation even in a single family. Among the Musaceae, *Heliconia* shows a narrow layer of functional perisperm around the endosperm; while in *Strelitzia*, the perisperm becomes reduced to a thin layer of broken down tissue.

Among the earlier writers who have dealt with the Polygonaceae, Strasburger (20), in 1879, described in detail the development of the mature embryo sac in *Polygonum divaricatum*, taking up the arrangement of the cells in the flower rudiments, the plan of the ovule and embryo sac and the development of the latter. He includes a brief description of the formation of the integuments and the course of the vascular bundle in the ovary and ovule, but says nothing regarding the morphology of the seed in its later development. In a later work (1902), Strasburger also simply describes *Polygonum orientale* with respect to the arrangement of the parts at the time of the mature embryo sac.

Dammer, writing on the Polygonaceae in Engler and Prantl (6), described the fruit and seed of Polygonaceae, dealing with the adaptations for dissemination, rather than the morphology of the seed.

The writer has made a careful study of certain genera of the Polygonaceae to determine if the absence of perisperm as a storage tissue noted in *Fagopyrum* by Stevens (1912), holds in the mature seed of other Polygonaceae. The genera under observation have been *Polygonum*, *Polygonella*, *Rumex*, and *Rheum*. In each of these genera, one or more representative species have been taken into consideration.

The material on which this study was made was collected during the summer of 1911 and 1912, and killed in weak chromo-acetic acid. Sections were cut about 12 μ in thickness and stained with Delafield's haematoxylon. An interpretation of the exact position and shape of the embryo in the mature seed was gained by free hand dissections under the binocular microscope.

A further study of the changes which occur in the seed tissues of *Fagopyrum*, *Polygonella*, *Polygonum*, and *Rumex* during the process of germination has also been carried on by the writer.

It was at the suggestion of Prof. A. W. Evans that this work was carried on, and it is to him that the writer is indebted for valuable assistance in all parts of the work, and to Dr. G. E. Nichols for liberal aid in the preparation of material.

Description and Discussion of the Morphology of the Seed

The growth and differentiation which take place in the ovules in response to the stimulus of sexual union have been studied in detail in the seeds of representative species in each of the genera *Polygonum*, *Rumex*, *Polygonella*, and *Rheum*, special emphasis being laid upon the morphology of the structures present in the mature seed.

Polygonum

P. Persicaria, *P. aviculare*, *P. tenue*, *P. sagittatum*, and *P. Convolvulus* were studied in detail, but as there was a marked similarity in their development it will be sufficient to describe in detail only one species, *e. g.*, *P. Persicaria.* The points of difference between this type and the remaining species mentioned will be taken into consideration.

The early development of the embryo in *P. Persicaria* up to the stage at which the cotyledons begin to be differentiated appears to correspond almost cell for cell with the typical *Capsella* embryo. The suspensor of *P. Persicaria*, however, consists of only two or three cells, whereas in *Capsella* it is made up of seven or eight cells.

Very soon after fertilization, free nuclear division begins in the embryo sac, and when the embryo has reached the octant stage, shown in *figure* 1, the cytoplasm of the endosperm containing about 30 free nuclei forms a granular layer, *EN*, lining the embryo sac, *K*. A single outermost layer of the nucellus, *N*, extending to the micropyle, differs markedly from the rest. At some period before fertilization occurs, this layer becomes differentiated and is made up of closely packed, prismatic cells, characterized by rather dense granular contents and an absence of vacuoles. The embryo sac, occupying a central position in the nucellar tissue extends nearly to the base. The micropylar portion of the sac is expanded being separated from the inner integument only by the differentiated nucellar layer.

As development proceeds growth and nuclear division occur most rapidly in the micropylar portion of the endosperm, in the region about the developing embryo. At about the time of the first indication of cotyledons in the embryo, cellular formation appears in this region, giving rise to a thin layer of cells around the embryo and also extending across the embryo sac below it. As further cell formation proceeds in the endosperm, a marked differentiation becomes apparent. At the stage shown in *figure* 2, the cell formation has proceeded until a portion of the endosperm, *EN*, about 6 cells thick extends across below the embryo. The endosperm above and at the sides of the embryo consists of a layer one or two cells thick, while below the thickest region, the endosperm again becomes gradually thinner and thinner, until at the base, for about one-fourth its length it no longer shows cell division but consists of a layer of nucleated cytoplasm, enclosing the large central sap cavity. This cytoplasmic layer merges into a cylindrical haustorium-like mass of dense protoplasm, *H*, at the base of the embryo sac, which apparently has no special function, as it undergoes very little change in the later development of the seed.

This marked differentiation of the endosperm into a cellular and non-cellular region, presented a condition similar to that described by Hofmeister (8, p. 185) and Strasburger (19, p. 101) in which the first division of the primary endosperm nucleus gives rise to a two chambered embryo sac, in only one of which the endosperm is developed.

A secondary differentiation in the cellular portion of the endosperm becomes evident very soon after the stage just described; the outermost layer assuming the appearance and function of a "cambium" layer which cuts off cells only from the inner side (*fig. 2a, CA*). Chamberlain (4, p. 344), in the developing endosperm of *Dioon edule*, and Stevens (18, p. 61), in *Fagopyrum esculentum*, have found similar conditions. These "cambium" cells divide rapidly, forcing the newly formed cells toward the center and downward as shown in *figure 2a*. The size of the sap cavity is thus diminished through the active growth of these cells. Very soon after the stage shown in *figure* 2, the cotyledons appear in the embryo, the sap cavity continuing to become smaller, until it becomes completely obliterated. As the embryo continues in development at the expense of the endosperm, it comes to lie in one angle of the seed with the faces of the cotyledons parallel with the flat surfaces of the seed as shown in *figure* 3. At this stage the endosperm cells differ considerably in appearance. The "cambium" cells and those adjacent are prismatic and densely filled with protoplasm, no intercellular spaces being present. Nearer the center the cells are larger, vacuolated, polyhedral and often binucleate, intercellular spaces also appearing. Numerous starch grains appear in the cells of this central tissue. Of the cells about the embryo only the distorted remains appear. Stevens (18, p. 63, fig. 4) has represented a similar condition for *Fagopyrum esculentum.*

The differentiated outermost layer of the nucellus has persisted in an actively growing condition up to this stage keeping pace with the growth of the ovule. The cells of this layer have not divided, but the individual cells, except at the micropylar region, have become tangentially enlarged and vacuolated. As the embryo approaches maturity, these cells become more and more coarsely vacuolate, until in the mature seed only the crushed remains of this layer are evident. Stevens, p. 63, has figured a similar condition for *Fagopyrum esculentum.* The micropylar portion of this layer is made up of a single plate of much thicker cells undergoing no very appreciable change after the time of fertilization. Practically all of the remaining nucellar tissue has been destroyed, only a comparatively few crushed and distorted cells remaining around the haustorium-like structure earlier mentioned (*fig.* 3, *N*).

The persistence of the differentiated outer layer in an actively growing condition during the entire endosperm development, and the fact that it is characterized by dense granular contents, suggests that it serves a nutritive function. Coulter and Chamberlain (5, p. 178) have termed this layer the "nutritive jacket." The inner integument usually gives rise to this layer; however, Billings (2, p. 278), as one exception, describes, in *Armeria plantaginea,* such a layer, which he terms "tapetum," as derived from the outer portion of the nucellus. Stevens (p. 62) has described in *Fagopyrum esculentum* a similar condition. Billings finds in *Erodium gruinosum* a "tapetum" made up of two layers, the inner one arising from the nucellus, and the outer from the integument.

Lloyd (13, p. 103) has shown that in the date, nutritive material is distributed to the developing endosperm through the integuments to some extent. In *P. Persicaria,* however, the integuments apparently undergo very little differentiation, remaining as rather thin uniform structures, each consisting of but two layers of cells. As the ovule of *P. Persicaria* is furnished with no vessels for the transfer of nutritive material from the chalaza to the growing endosperm, it is quite conceivable that the differentiated outer layer of the nucellus assumes this function. Further, the more rapid endosperm development in the micropylar region is evidently due to the fact that food material is brought there by way of this layer, since the broken-down cells of the undifferentiated nucellar tissue form a barrier to any passage of food substance through that channel.

As the seed approaches maturity, a further differentiation occurs in the endosperm. After the "cambium" layer ceases cutting off starch-storing cells, it divides further by anticlinal walls; thus giving rise to a layer of short, thin-walled prismatic cells, which, in the micropylar region, undergo further division, developing to the exclusion of the starch-storing endosperm and passing abruptly into the latter. At the maturity of the seed, shown in *figure* 4, the cells of this layer, *A*, are densely filled with granular contents, but contain no starch. This is an aleurone layer, the "eiweisshaltige Zellen" of Harz. Except for this aleurone layer the remaining portion of the endosperm, *EN*, consists of large, thick walled cells, closely packed and filled with starch grains, which by their pressure cause the nuclei to become flattened and irregular in outline.

In the mature seed, which is 2 mm. wide and 2.5 mm. long, the cotyledons of the embryo are flattened and slightly broader than the hypocotyl. The entire embryo occupies a cavity at one side of the seed (*figs*. 4, *E*, and 9, *C*), this cavity having been formed by the continuous growth of the embryo at the expense of the endosperm. The cells lining this cavity are somewhat broken down and irregular in outline due to their depletion by the developing embryo.

The only vestige of the original nucellus present in the mature seed as perisperm is a mass of broken-down tissue at the base of the seed (*fig*. 4, *N′*) and the crushed remains of the nutritive layer. It is interesting to note that during the later development of the seed these tissues have apparently served no function, and do not represent a storage region, this function being entirely carried out by the endosperm with its aleurone layer.

The thin, uniform integuments, in the development of the seed to maturity, have become closely compressed, so that only their crushed remains appear in the mature seed (*fig*. 4, *I*).

The seed, at maturity is enclosed in a thick hard coat, consisting of the cutinized epidermal layer and the compressed inner layers of the ovary (*fig*. 4, *O*).

If attention is now turned to a consideration of *Polygonum aviculare* and *Polygonum tenue*, it is seen that the development of these two species appears practically identical, therefore, the variations from the type will be followed only in *Polygonum aviculare*. The early stages are essentially the same, *figure* 1 representing very well the condition at the octant stage for both species. The nucellus of *P. aviculare*, however, becomes degenerated to a slightly greater extent. As growth proceeds beyond this point, the method of development is the same but the rapidity of endosperm formation is much greater. At the time of the first indications of cotyledons in the embryo, the embryo sac is already filled with cellular endosperm, and the nucellus has been reduced to a few crushed cells at the base of the seed, and the nutritive layer. The haustorium-like mass of protoplasm at the base of the seed is much broader. The suspensor of the embryo is made up of four cells, instead of only two as in the majority of seeds of *P. Persicaria.*

The later development follows much the same plan, the growing embryo coming to lie in one of the corners of the seed. At maturity (*figs.* 5 and 8), the seed is 2.35 mm. long and 1.26 mm. wide. The embryo is longer than in *P. Persicaria*, and extends across the chalazal region. The cotyledons are semicircular in outline (*fig.* 8, *C*) and the cotyledon away from the starchy endosperm lies with its back against the chalazal region of the seed. As growth has proceeded, the cotyledon next the endosperm has grown down over the other cotyledon. The cylindrical, massive hypocotyl is the same width as the cotyledons at the base of which appears a well-developed, cylindrical plumule (*fig.* 5, *P*).

Polygonum Convolvulus and *Polygonum sagittatum* agree in most respects with the type. At the octant stage of *P. Convolvulus* (*fig.* 6), however, the ovule is much broader and the integuments relatively thicker than in *P. Persicaria.* At maturity, the cotyledons are rather broad and flattened, the edges pressing against the base of the seed with the tips well past the middle line of the seed. The position of the cotyledons with respect to the chalazal region of the seed agrees with the condition found in *P. Persicaria*, but differs from that found in *P. aviculare* and *P. tenue*, in which the back of the outer cotyledon lies pressed against the chalazal region of the seed. *Figures* 7 and 10 represent the mature condition found in *P. Convolvulus*, and may also serve the same purpose for *P. sagittatum*, except that in the latter species the cotyledons are somewhat broader.

Rumex

In this genus, the two species studied, *Rumex crispus* and *Rumex Acetosella*, are essentially alike in their development, and follow to maturity the same plan as that described for *Polygonum Persicaria*. Some interesting differences from the type appear in the mature seeds.

The embryo of *R. crispus*, in its development to maturity, comes to lie usually at one side of the seed (*figs*. 11, *E*, and 13, *C*) with the back of the outer cotyledon pressed against a flat surface of the seed and the chalazal region. The massive, somewhat flattened hyocotyl is the same width as the cotyledons which are semicircular in section. The mature seed is 2.45 mm. long and 1.55 mm. wide.

In *Rumex Acetosella* the mature embryo (*fig*. 12, *E*) usually occupies the same relative position at one side of the seed as in *R. crispus*. The embryo is very much more curved, corresponding with the shorter and relatively broader seed, which is 1.2 mm. long and .9 mm. wide. The cotyledons, similar in section to those of *R. crispus* (*fig*. 13, *C*), extend across the chalazal region almost to the angle opposite.

The position of the embryo against a flat surface of the seed is apparently not a fixed character in *Rumex*, for the writer in examining a number of seeds of both the above species, found, in several cases, the embryo situated in an angle of the seed. This is a marked contrast to the conditions found in the genus *Polygonum*, for there the position of the embryo seems to be a fairly fixed character.

Polygonella

The single species studied, *Polygonella articulata*, showed some very marked differences from the selected type, *Polygonum Persicaria*, although some stages in the development correspond very closely.

The early development up to the octant stage agrees with the type, except in the length and structure of the suspensor. At this stage, shown in *figure* 14, the suspensor, *T*, is about two thirds the length of the embryo sac, and is made up of large inflated cells. The lower two thirds consists of two rows of cells, while the upper micropylar portion is made up of a single chain of four or five cells.

As development proceeds growth and nuclear division in the endosperm is uniform in the micropylar region, and about the periphery of the embryo sac. At about the time that the meristematic regions become differentiated in the embryo, cell formation appears in the free endosperm, giving rise to the uniform layer of cellular endosperm. At the stage shown in *figure* 15, in which the cotyledons are becoming differentiated, this endosperm layer, *EN*, has become two cells thick, the outermost layer being differentiated into a "cambium" as in *Polygonum Persicaria*. The suspensor has become somewhat shriveled and the embryo imbedded in the layer of cellular endosperm lining the embryo sac.

The growth of the endosperm from the stage just described proceeds as in *Polygonum Persicaria*. At the stage shown in *figure* 16, the embryo is situated well down toward the base of the seed. The space about the embryo is continuous with a long narrow cavity, *CY*, extending to the micropyle. This cavity contains the shriveled remains of the suspensor and serves as a path for the later elongation of the hypocotyl. *Figure* 17 shows a later stage in which the hypocotyl and fleshy cotyledons have become quite well differentiated, and the cavity containing the shriveled suspensor has become somewhat larger.

The further growth and differentiation of the various tissues of the ovule proceed as in the type. The mature seed (*figs*. 18 and 19) which is 1.8 mm. long and .8 mm. wide, presents some differences in the tissues formed. At the micropylar portion of the aleurone layer (*fig*. 18*a*, *A*) appears an outer region consisting of prismatic cells containing dense granular contents, and an inner region consisting of irregular cells filled with a watery solution. The depleted condition of these cells has evidently been brought about through absorption by the surrounding tissues. The embryo, in its mature condition, is usually situated in an angle of the seed (*fig*. 19, *C*). The position of the embryo in this species seems to be quite as unstable a character as in *Rumex*, for, in a number of cases examined, the mature embryo occupied a position against one of the flat surfaces of the seed.

The relatively long hypocotyl passes into the broader, fleshy cotyledons, which are elliptical in cross section and lie pressed against the base of the seed, with their faces at an angle of about 45 degrees to a plane cutting the long axis of the seed at right angles (*fig*. 18, *C*).

This position of the cotyledons is in marked contrast to the condition found in *Polygonum* and *Rumex*. In *P. Persicaria*, *P. Convolvulus*, and *P. sagittatum*, the edges of the cotyledons are pressed against an angle of the seed and the chalazal region, while in *P. aviculare*, *P. tenue*, *Rumex crispus*, and *R. Acetosella*, the back of the outer cotyledon presses against a face of the seed and the chalazal region.

Rheum

A single species, *R. Rhaponticum*, was taken into consideration. Its development to maturity corresponds in many respects to that of *Polygonum Persicaria*, but some differences are evident, which deserve special attention.

The mature fruit is broadly three winged, a feature quite characteristic for this genus. The length of the fruit is 5 mm. and the width, exclusive of the wings, 3.6 mm. The inner wall of the ovary so indents the testa of the seed as to cause it to be very much longitudinally folded, as shown in *figure* 21.

The aleurone layer (*fig.* 20, *A*) instead of being several cells thick, as in the type, consists of a single layer of cells. The mature embryo, occupying a central position (*figs.* 20 and 21) is relatively larger than the type, corresponding with the larger size of the seed. Stevens found the mature embryo of *Fagopyrum esculentum* also occupying a medium position in seed. *Rheum* and *Fagopyrum* are the only Polygonaceae according to the investigations up to the present time, possessing this character. The mature seed of *R. Rhaponticum* is abruptly narrowed at the apex, so that the short, massive, cylindrical hypocotyl practically fills the micropylar region of the seed, only the aleurone layer separating it from the testa (*fig.* 20). In *R. palmatum*, Lubbock (14, p. 436) found a similar condition, but in *R. officinale*, he found the seed not abruptly narrowed at the apex. The broad, thin cotyledons (*fig.* 21, *C*), which are about two and one half times the length of the hypocotyl, do not extend to the base of the seed, as shown in *figure* 20. They are sometimes more or less folded longitudinally. Lubbock describes an exceptional condition in *R. palmatum* in which the cotyledons are oblique to one another, and in one instance they were twisted at right angles to one another so as to occupy the three angles of the seed and thus attain the greatest possible size.

A very interesting feature in *R. Rhaponticum* is the petioled condition of the cotyledons at the base of which is a quite well developed plumule. In *Polygonum*, *Polygonella*, and *Rumex*, the cotyledons spring directly from the hypocotyl without developing distinct petioles.

Germination of Certain Polygonaceae

These investigations were carried on in order to gain a knowledge of the fate of the embryo, starchy endosperm, and aleurone layer in the mature seed during germination. Most of the seeds were germinated between wet filter papers, a few, however, were placed in the soil. The latter were used in determining the relation of the very young seedling to the surface of the soil. The material, on which the study of the changes occurring in the germinating seeds was carried on, was cut in sections 18–22 μ in thickness and stained with Delafield's haematoxylon and orange G.

Rumex crispus

The seed shows no external change during the first two days, but significant internal changes are occurring. The cells of the thickened micropylar region of the aleurone layer first show very great activity. In the ungerminated seed the entire layer is made up of short prismatic cells, containing large nuclei and filled with deeply staining cytoplasm. The cells of the micropylar portion now become much enlarged and numerous fine granules appear scattered quite uniformly through the fine alveolar cytoplasm. In certain seeds the granules were surrounded by a clear liquid. This increase in size of the cells is brought about not only by osmotic pressure but also by an actual increase of cell wall structure.

It is quite probable that the occurrence of these granules in the cytoplasm of the aleurone layer is associated with the secretion of a digestive ferment as is true in the digestive cells of certain other seeds and in the digestive glands of animals. Brown and Morris (3), through observation on the changes in the scutellar epithelium of the barley grain, find that their thin-walled, columnar cells in the process of germination undergo a significant change. Before germination begins, the cell contents are finely granular and the large, elliptical nucleus lies near the base of the cell with its longer dimensions across the cell. Within a few hours after germination begins the very fine granules in the protoplasm become much larger and coarser and increase in number to such an extent that the formerly conspicuous nucleus is almost obscured. This granularity is maintained until the endosperm is almost exhausted of its reserve materials, then the protoplasm becomes clear and transparent, the nucleus having disappeared.

Torrey (22), working on the cytological changes accompanying the secretion of diastase in the epidermal cells of the scutellum in maize, finds granules appearing in the cytoplasm during germination very similar to the condition found by Brown and Morris in the diastase secreting cells in the barley. In both these cases the nucleus finally disappears when the cell has exhausted itself after long continued secretion. In *Rumex* the writer was unable to follow the earlier changes undergone by the nucleus; however, it was noted that the nuclei lose their staining power and finally disappear as the secreting cells become exhausted. It is, nevertheless, quite probable that the nuclei do play an important role in the production of the ferment. Torrey gives a very interesting interpretation of the origin of these granules in the epithelial cells of the scutellum in maize. At the beginning of germination the nuclei of these cells contain dark staining granules, which are ejected into the cytoplasm through minute breaks in the nuclear membrane. These granules at first spread through the cell, but later collect at the end of the cell next to the endosperm, where they are ultimately dissolved. In *Rumex* the granules are distributed quite uniformly through the cytoplasm of the secreting cells and no such origin as Torrey describes is evident. He also notes an enlarged condition of the secreting cells similar to that which occurs in *Rumex*. He evidently considers this enlargement to be due merely to osmotic pressure without an increase of cell wall structure as the writer believes to be the case in *Rumex*. He suggests (p. 430) that the osmotic activity is very likely set up by some substance secreted in the cell at this time. The protein nature of diastase prevents it from being as osmotically active as other less complex substances, as for instance organic salts or acids, which when present in the cell exercise a strong osmotic attraction. Nevertheless, the enlargement of the cell occurs when the nucleus has become completely filled with these granules. The formation of some organic acid during the great metabolic activity of the cell may be a possible explanation of this phenomenon. This hypothesis is further supported by the fact that diastase, to be especially effective as a ferment in physiological experiments, must be dissolved in a slightly acid liquid.

In contrast to the condition found in the plant kingdom, Mathews

(15) has found in the animal kingdom that the nucleus of the pancreatic cell does not play an active role, but indirectly controls the zymogenesis. Haidenhain and Korshelt consider that the changes in position and size of the nuclei during secretion are signs of functional nuclear metabolism.

There is associated with the increase in size and granularity of the aleurone cells in *Rumex* a gradual change in the endosperm and embryo. This change is seen in the endosperm as a slight depletion of the starch cells next to the thickened micropylar portion of the aleurone layer and next to the embryo. Perhaps the depletion in the latter region is entirely due to a ferment secreted by the embryo itself. Newcombe (16, p. 71) has found in the buckwheat that the cotyledons do actually secrete a ferment which has a weak solvent power on wheat-starch grains, and on the membranes of the barley endosperm. This may be true for *Rumex* but it seems more probable that the aleurone cells, which at this time have every appearance of active digestive cells, are secreting an enzyme which is the principal agent in the dissolution of the starch in the endosperm. This enzyme diffuses to the starchy endosperm in the watery solution about the embryo. As a result of the action of the enzyme on the starch, there is a conversion of the insoluble starch into a soluble form, in which form it is absorbed by the embryo. In connection with the transfer of the dissolved starch to the germinating embryo an interesting phenomenon may be noted. As the carbohydrate solution is absorbed by the growing embryo, instead of being entirely consumed at once by the growing embryo, as is assumed to be the case in endosperm-containing seeds, a part of this absorbed solution is converted back into the insoluble form and stored in the cells of the embryo in the form of compound starch grains. The depletion of the starch cells continues, consecutively towards the outside of the seed, the depleted endosperm cells in contact with the embryo becoming collapsed by the pressure of the latter. As the micropylar portion of the aleurone layer becomes exhausted through continued secretion, the basal thinner portion comes into activity, each individual cell acting independently of those adjacent.

If attention is now turned to a consideration of the changes in the embryo associated with the activity of the aleurone layer and the dissolution of the starch, it is seen that important changes are taking place. There occurs a rapid elongation of the embryo. This elongation forces the coat of the fruit apart at the apex, the break appearing at the angles of the fruit. As the embryo increases in size, the tip and then the whole radicle appears outside the fruit, as shown in *figure* 22. In so doing the micropylar portion of the shriveled testa, *D'*, is either pushed aside or carried outside a short distance with the radicle, *R*. As the radicle increases in length, the increase in size of the aleurone cells mentioned above, causes the micropylar portion of the layer, *L*, to appear outside the fruit and clasp the radicle, thus preventing any of the dissolved food material escaping from the seed in its passage from the endosperm to the embryo. This collar-like structure must also prevent to a great extent the entrance of water from without, which would dilute this food material, or bacteria and fungi which would cause its destruction.

As the radicle of the seedling continues to elongate it grows downward (*fig.* 23, *R*) and enters the soil, there becoming anchored by numerous root hairs and secondary roots. There occur at the same time, an elongation and broadening of the cotyledons, and a slight differentiation of the stalks. As germination continues the basal portion of the cotyledons emerges from the seed (*fig.* 24). By this time, the plumule which was hardly differentiated in the mature embryo, has attained a length of about 2 mm. and is cylindrical in form (*fig.* 24, *P*). The vascular system of the young seedling, which was undifferentiated in the mature embryo, has now become quite clearly differentiated. As the elongation of the cotyledons is occurring, the hypocotyl increases in length, raising the cotyledons with the seed adhering to the surface of the soil. At the stage shown in *figure* 24 the starch has all been absorbed from the starchy endosperm except within the regions included by the dotted line. The depleted cells retain practically their normal shape, only being broken down as the cotyledons increase in size and press against them. The inactive aleurone cells have lost their contents and their walls have become collapsed. Very soon after this the broadening cotyledons, which have now developed chlorophyll push off the wasted remains of the seed and become erect.

Fagopyrum esculentum

As the writer has not included a representative of the genus *Fagopyrum* in the morphological discussion of the Polygonaceae, a description of the mature fruit of the buckwheat, *F. esculentum*, will be given at this time.

The mature fruit is an achene, triangular in cross section and brownish black in color. The seed is large, filling the cavity of the ovary and conforming to its shape. The broad, thin cotyledons of the embryo are variously oriented from one angle of the seed to another, and present in cross section the appearance of a much distorted letter S. *Figures* 25 and 26 will give some conception of the complicated form of these cotyledons. The shortly petiolate cotyledons follow the interior of the testa for some distance outside the starchy endosperm, in close contact with the aleurone layer, which is uniformly one cell thick as in *Rheum Rhaponticum.* The cotyledons lie face to face with their midribs together and the width of the blade on one side of the midrib of each cotyledon is about twice that of the other side. As a result of this unsymmetrical form of the cotyledons, one of them extends beyond the other, the smaller half of each cotyledon being enclosed by the corresponding half of the other. In the mature fruit the radicle is rather large and tapers to an obtuse point, being completely enclosed by the basal portion of the cotyledons. The starchy endosperm is exceedingly irregular as a result of the peculiarly unsymmetrical nature of the embryo. The cotyledons do not extend quite to the base of the embryo sac in the mature seed according to Stevens, a space containing the remains of the undeveloped basal portion of the embryo sac, being always present in the chalazal region of the seed. In the mature seed the embryo has a well-differentiated vascular system, and the cotyledons have become differentiated into epidermal, palisade, and spongy tissue as well as conductive tissue.

The germination of *Fagopyrum esculentum* presents some very distinctive features although in some respects there is a marked similarity to the conditions found in *Rumex crispus.*

During the first two days there is very little external change except a slight enlarging of the seed. Internal changes are however very likely occurring for very soon the apex of the fruit becomes split along its angles. The aleurone layer shows marked activity in the enlarging of the cells especially in the micropylar and basal regions. As this enlargement occurs, the cytoplasm of the cells becomes alveolar and fine granules appear quite uniformly distributed through it much as in *Rumex crispus.* It is quite probable that there is associated with this activity of the aleurone layer, as suggested for a similar condition in *Rumex*, a secretion of a ferment which dissolves the starch of the endosperm. The digestion of the starch proceeds much as in *Rumex.*

The first evidence of the action of the ferment is seen in the depletion of the starch in the micropylar and basal regions. The dissolved starch is apparently not used up as fast as it is absorbed by the embryo but rather a part of it becomes converted back into an insoluble form in the cells of the embryo as already noticed for *Rumex crispus*. The depletion of the endosperm in *Fagopyrum* proceeds much as in *Rumex*.

As the starchy endosperm is being digested and absorbed by the embryo, the latter begins to increase in size. Very soon through the rent at the apex of the fruit the elongating hypocotyl and radicle appears outside the seed (*fig.* 25). The enlarged aleurone layer does not extend beyond the coat of the fruit as is the case in *Rumex*.

In sections of the seed at the stage in which the radicle is just appearing outside the seed, the elongated tannin sacs, mentioned by Solereder (17, p. 669) as occurring in species of *Fagopyrum* and *Polygonum*, are very evident. As further growth occurs these sacs increase markedly in length. Another interesting feature in the young radicle is the embryonic secondary roots which appear under the microscope as distinct slight elevations of the surface of the radicle.

As these changes are taking place in the hypocotyl and radicle, the cotyledons are increasing quite markedly in width and length, and to a slight extent in thickness. This increase in proportions is doubtless due to cell divisions as well as increase in cell size. The petioles or stalks of the cotyledons, which are so short in the ungerminated seed that they can be hardly distinguished as such, take on a slow growth as germination continues. In the stage shown in *figure* 25, they are only about .2 mm. in length. At about the fourth day of germination these stalks have become about 1 mm. in length and at their basal portion, merge into a swollen sheath-like structure of equal length (*fig.* 27, *SH*) which protects the developing plumule. As growth proceeds the basal portion of the cylindrical cavity of the sheath becomes lined in the region of the plumule with multicellular hair-like structures. The shape of the plumule (*fig.* 27, *P*) which is now about 4 mm. in length and almost fills the basal portion of the cavity, is that of an inverted top.

At the stage just discussed which is shown in *figure* 27, the basal portion of the cotyledons are loosely folded about the hypocotyl, which has now increased quite markedly in diameter. The increase in width of the cotyledons causes them to become much more folded than originally, and also causes the blades to extend nearly the whole distance around the inside of the seed as shown in *figure* 26. The starchy endosperm and the digestive aleurone layer have now become almost entirely depleted and the pressure of the enlarging cotyledons has collapsed the cells. Only the broken down remains of the aleurone layer are present inside the seed coat; and in the folds of the cotyledons at their basal portion, appear the distorted, empty starch cells (*fig.* 27, *EN*). The rapidly enlarging cotyledons soon push off the wasted remains of the seed and unfold, becoming oriented in a horizontal position. These cotyledons present now very much the appearance of regular foliage leaves; they are equipped with chlorophyll and all the tissues typical of foliage leaves.

Polygonella articulata

An attempt made in the early part of March to find some of the seeds in the sand about the dead plants resulted in the finding of only already germinated seeds. The hypocotyl and radicle had attained a length of about 12 mm. A number of the seedlings were allowed to develop further in the laboratory in order to make a study of the later stages in germination.

Upon examination of the earliest stages available conditions were found quite similar to those already described for *Rumex crispus*. At the stage shown by *figure* 28, the entire aleurone layer has become greatly enlarged. The cells in the thickened micropylar region show the characteristic granules of the digestive layer, while the remaining cells show a loose alveolar cytoplasm entirely free from granules. The enlarged condition of the aleurone cells causes the micropylar region of the layer to appear outside the seed coat (*fig.* 28, *L*) forming a collar-like structure as in *Rumex crispus*. This digestive layer behaves in a manner similar to that described in the preceding example. In the digestion of the starch there appears a marked difference, the starchy endosperm being depleted of its contents quite uniformly, instead of being progressive in its depletion. In contrast to *Fagopyrum* and *Rumex*, the digestive starch is all used up by the germinating embryo as it is absorbed, none of it is converted back into an insoluble form as in the above species. This digestion proceeds much slower than in the other species studied, consequently there is a corresponding slow growth of the young seedling.

As this growth proceeds the cotyledons gradually increase in length and become quite fleshy. The increase in size crushes the depleted starch cells and when the germination has reached the stage shown in *figure* 29, only the crushed remains of the endosperm and aleurone layer are present in the seeds. By this time the plumule, *P*, has increased in size, having attained a length of about 1 mm. and a width at its base of about .06 mm. It is conical in shape, the apex being slightly rounded. No multicellular hair-like structures are present about the plumule as in the preceding examples. Very soon after this the broadening cotyledons push off the seed remains, and become oriented in a nearly horizontal plane. The differentiation of the cotyledons into stalk and blade is only slightly evident at this time. Very little differentiation has occurred in the tissues of the cotyledons with the exception of the epidermis which has now become well developed.

Polygonum scandens

The ungerminated seed is very similar to that of *P. Convolvulus* already described in this paper. In following out the germination, conditions are found to be practically the same as described above for *Rumex crispus*, so a detailed discussion of the processes involved need not be entered into. A comparison of *figures* 23 and 24, representing certain stages in the germination of *Rumex crispus*, with *figures* 30 and 31, representing corresponding stages in *P. scandens*, shows how very similar in appearance are the methods of germination in the two species.

Systematic Relation of the Polygonaceae

Bentham and Hooker (1), include under the Curvembryeae, the Nyctaginaceae, Illecebraceae, Amaranthaceae, Chenopodiaceae, Phytolaccaceae, Batidae, and Polygonaceae. In these families the authors speak of the storage tissue outside the embryo as "albumen," without stating whether this tissue is endosperm or perisperm.

The writer sees no reason to doubt that Dammer, writing on the Polygonaceae in Engler and Prantl (6), has properly taken out the family from the Curvembryeae of Bentham and Hooker and placed it in a separate group termed Polygonales. Harz (7) agrees with Bentham and Hooker in placing the Polygonaceae in the Curvembryeae, and looks upon the storage tissue outside the embryo as perisperm rather than endosperm. This misinterpretation of the structure in the mature seed doubtless led him to place the Polygonaceae among the families characterized by perisperm containing seeds and curved embryos, comprising the Caryophyllaceae, Paronychiaceae, Portulaceae, Phytolaccaceae, Sclerenthaceae, Chenopodiaceae, Amaranthaceae, and Nyctaginaceae. In discussing the Curvembryeae, Harz states that he finds without exception in all the species studied an oily endosperm in slight amounts laid down about the axis of the embryo so that the radicle is surrounded by an endosperm sheath. The observations of the writer show that this structure noted by Harz is not so specialized a character as he implies, but rather represents the micropylar portion of the aleurone layer which is also continuous to the chalazal region of the seed.

Since the Polygonaceae are characterized by an abundant endosperm instead of perisperm as believed by early writers, there cannot be the close relationship between the Piperaceae and Polygonaceae that Johnson (11, 1902) suggests.

In his work on Seedlings, Lubbock (14) has followed the classification given by Bentham and Hooker and correctly interpreted the storage tissue in the seed of Polygonaceae as endosperm. Neither in the Polygonaceae nor in any of the other families which he included under the Curvembryeae does he refer to the aleurone layer of the endosperm or to the perisperm.

Among the Dicotyledons having a curved embryo and endosperm, the Nolanaceae, Solanaceae, Resedaceae and Convolvulaceae may be mentioned, but in each case there are other more prominent characters which cannot be overlooked and prevent them from being considered close relatives of the Polygonaceae.

The variations in the morphology of the Polygonaceae seed would seem to show that this family represents a more or less plastic evolutionary line. A primitive character is seen in case of the median embryo of *Rheum* and *Fagopyrum*, and a marked advance over this type in the curved embryo of *Polygonum*, *Polygonella* and *Rumex*. The orthotropous ovule prevailing in this family is also a primitive character. If some of the other characters of the Polygonaceae, as the wings developed on the fruit of *Rheum*, and the ochrea or tubular stipule occurring all through the family, are taken into consideration it is very evident that there has occurred some very high specialization.

SUMMARY

The outermost layer of the nucellus becomes transformed into a nutritive jacket at some period before fertilization. This layer apparently carries food material from the chalazal region to the developing endosperm. The remaining nucellar tissue becomes broken down comparatively early.

In *Polygonum*, *Rumex*, and *Rheum*, the cellular endosperm appears first in the micropylar region, while in *Polygonella* it appears uniformly about the periphery of the embryo sac. The cellular endosperm increases in extent by the activity of a cambium-like layer differentiated very soon after cell formation appears. Some time before maturity of the seed this "cambium" layer ceases cutting off cells and becomes differentiated into an aleurone-containing layer, which, in *Polygonum*, *Rumex*, and *Polygonella*, through further tangential cell division, becomes several cells thick in the micropylar region.

In *Polygonum*, *Rumex*, and *Rheum*, the suspensor is from two to four cells in length, while in *Polygonella* it is about twelve cells in length, the portion next to the embryo consisting of two rows of cells.

The embryo in its development to maturity comes to occupy a position either in the axis of the seed, an angle of the seed, or against one of the faces of the seed. The first position is seen in *Fagopyrum esculentum* and *Rheum Rhaponticum;* the second in *Polygonum Persicaria*, *P. Convolvulus*, *P. sagittatum*, *P. aviculare*, *P. tenue*, and *Polygonella articulata;* the third in *Rumex crispus* and *R. acetosella*. Only in *Polygonum*, *Fagopyrum*, and *Rheum* is the position of the embryo a fixed character.

The germination of *Rumex crispus*, *Fagopyrum esculentum*, *Polygonella articulata* and *Polygonum scandens*, shows that the aleurone layer has presumably a digestive function, secreting a ferment which converts the insoluble starch of the endosperm into a form available for the germinating embryo. The cells of the layer become much enlarged, and their cytoplasm finely alveolar and granular. In *Rumex*, *Polygonella*, and *Polygonum* this increase in size of the aleurone layer causes the micropylar portion to appear outside the seed coats.

In *Rumex* and *Fagopyrum*, the absorbed carbohydrate is temporarily converted back into starch in the tissues of the germinating embryo, the cotyledons being the principal storage region.

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EXPLANATION OF PLATES XLV–XLVIII

All *figures* drawn from median longitudinal sections, except *figures* 8, 9, 10, 13, 19, 21, and 26, which are from cross-sections. The following abbreviations are used: *A*, aleurone layer; *C*, cotyledons; *CA*, embryonic cells of endosperm; *CY*, cavity left by shriveled suspensor; *D*, depleted portion of aleurone layer; *D'*, remains of micropylar portion of integuments and nutritive layer; *E*, embryo; *EN*, endosperm; *F*, remains of suspensor; *H*, haustorium-like structure; *HY*, hypocotyl; *I*, integuments; *K*, embryo sac; *L*, enlarged aleurone layer; *N*, nucellus; *N'*, remains of undifferentiated nucellus; *NU*, "nutritive jacket"; *O*, mature seed coat; *OT*, mature seed coat plus remains of integuments; *P*, plumule; *R*, radicle; *SH*, sheath-like structure; *T*, suspensor.

FIG. 1. *Polygonum Persicaria*, showing the embryo in the octant stage and the endosperm in the free nuclear condition; × 53.

FIG. 2. *P. Persicaria.* The embryo showing the first differentiation of the cotyledons; the upper portion of the endosperm has become cellular, while no cell walls have appeared in the lower portion; haustorium-like structure evident at the base of the embryo sac; × 40.

FIG. 2*a*. Portion of longitudinal section of *P. Persicaria* seed at about the stage shown in *fig.* 2; only the outermost layer of the nucellus, the "nutritive jacket," remains functional; the endosperm shows an outermost layer of embryonic cells and a more central region of large vacuolate cells; × 248.

FIG. 3. *P. Persicaria.* A later stage in which the embryo sac has become completely filled with cellular endosperm, except at the base where the haustorium-like structure is seen; embryo at the side; × 31.

FIG. 4. *P. Persicaria.* Mature seed; endosperm filled with starch; aleurone layer present; nucellar tissue practically all obliterated; embryo at the side; dotted line indicates plane of cross section shown in *fig.* 9; × 31.

FIG. 5. *Polygonum aviculare.* Mature seed; aleurone layer present; endosperm filled with starch; embryo with well developed plumule at the side of the seed; nucellar tissue nearly obliterated; dotted line indicates plane of cross section shown in *fig.* 8; × 31.

FIG. 6. *Polygonum Convolvulus*, showing embryo in the octant stage and the endosperm in the free nuclear condition; × 64.

FIG. 7. *P. Convolvulus.* Mature seed; aleurone layer and starch filled cellular endosperm present; embryo at the side; nucellar tissue practically obliterated; dotted line indicates plane of cross section shown in *fig.* 10; × 20.

FIGS. 8–10. Fig. 8. *Polygonum aviculare;* fig. 9, *P. Persicaria;* fig. 10, *P. Convolvulus;* showing the relative position of the cotyledons in the three species; dotted lines indicate plane of longitudinal sections shown in *figs.* 4, 5, and 7; × 14.

FIG. 11. *Rumex crispus.* Mature seed; aleurone layer and starchy endosperm present; embryo at side of seed; nucellar tissue practically obliterated; dotted line indicates plane of cross section shown in *fig.* 13; × 31.

FIG. 12. *Rumex Acetosella.* Mature seed; starchy endosperm and aleurone layer present; embryo at side of seed; nucellus nearly obliterated; × 42.

FIG. 13. *Rumex crispus.* Cross section of mature seed showing position of cotyledons; dotted line indicates plane of longitudinal section shown in *fig.* 11; × 14.

FIG. 14. *Polygonella articulata*, showing the embryo in the octant stage supported by the characteristic long suspensor; endosperm in free nuclear condition; × 31.

FIG. 15. *P. articulata.* A little later stage in which the endosperm has become cellular except for the haustorium-like structure at the basal portion; embryo imbedded in the cellular endosperm; × 31.

FIGS. 16 AND 17. *P. articulata.* Successive stages in which the cotyledons have become well developed and the embryo sac practically filled with cellular endosperm; nucellar tissue reduced; × 31.

FIG. 18. *P. articulata.* Mature seed, showing cotyledons in section; aleurone layer, starchy endosperm, broken down nucellar tissue, and haustorium-like structure present; dotted line indicates plane of cross-section shown in *fig.* 19; × 31.

FIG. 18*a*. *Polygonella articulata*, showing detailed structure of micropylar portion of seed at stage shown in *fig.* 18; aleurone layer differentiated into a depleted and non-depleted region; starchy endosperm packed full of starch grains; × 163.

FIG. 19. *P. articulata*, showing position of cotyledons in the seed; dotted line indicates plane of longitudinal section shown in *fig.* 18; × 14.

FIG. 20. *Rheum Rhaponticum.* Mature seed; starchy endosperm and aleurone layer present; remains of nucellar tissue present at base of seed; dotted line indicates plane of cross section shown in *fig.* 21; × 14.

FIG. 21. *R. Rhaponticum*, showing position of the embryo in the seed; dotted line indicates plane of longitudinal section shown in *fig.* 20; × 14.

FIGS. 22–24. *Rumex crispus*, showing successive stages in the germination, in which the radicle of the young seedling appears through a rent in the micropylar portion of the seed and is clasped by the enlarged aleurone layer which appears outside the seed coat; × 20.

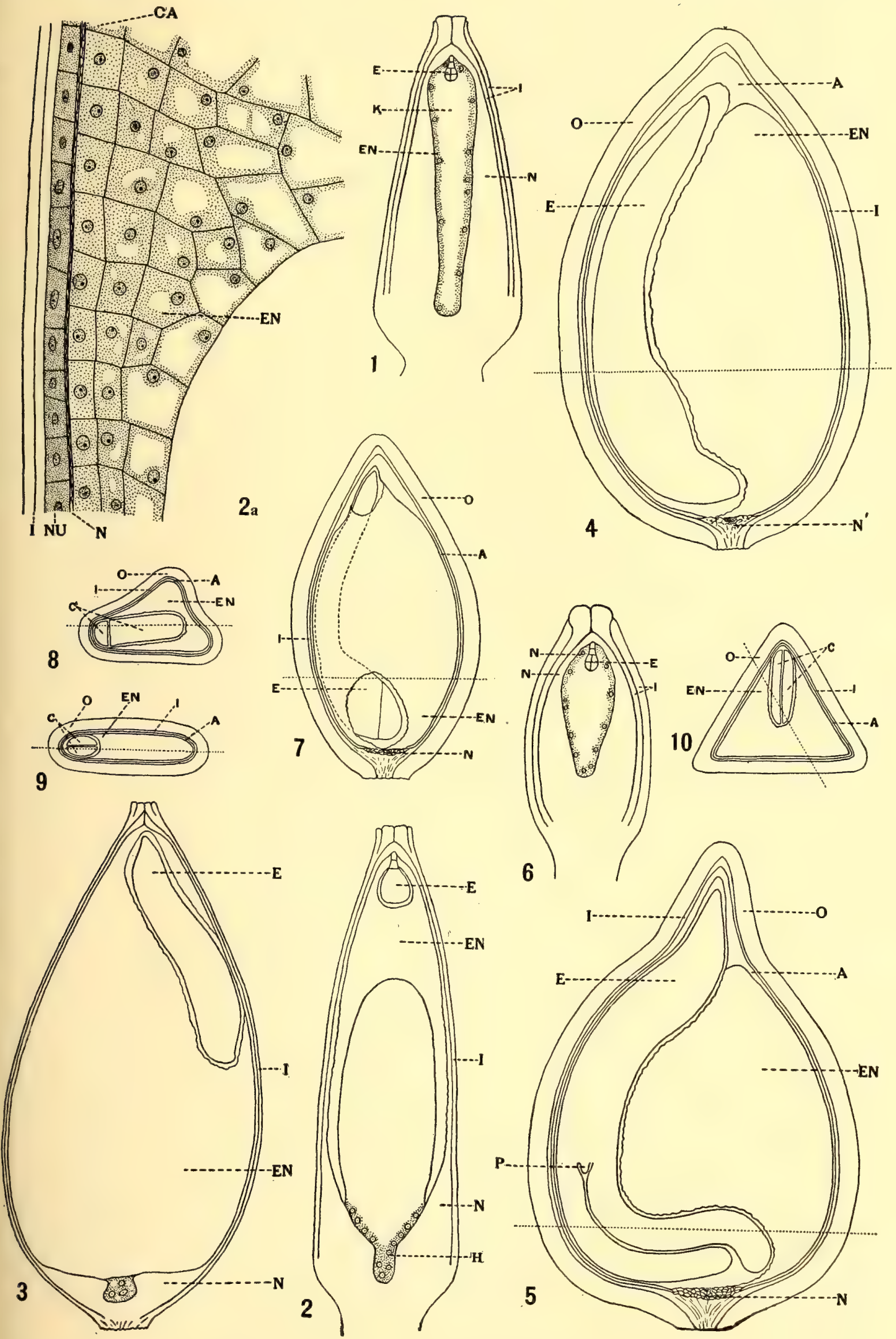
FIG. 25. *Fagopyrum esculentum*, showing an early stage in germination; × 14.

FIG. 26. *F. esculentum.* Cross section of seed at a stage somewhat later than that represented by the preceding figure; × 31.

FIG. 27. *F. esculentum.* Late stage in germination; seed remains almost ready to drop off the cotyledons; × 14.

FIGS. 28 AND 29. *Polygonella articulata.* Successive stages in germination; enlarged micropylar portion of the aleurone layer appears outside of seed coat and clasps the young seedling; × 31.

FIGS. 30 AND 31. *Polygonum scandens.* Successive stages in germination similar to those of *Rumex crispus* shown in *figs.* 23 and 24; × 14.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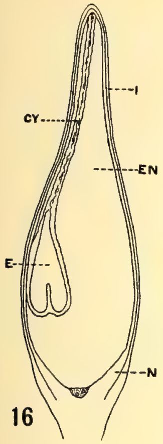
WOODCOCK: SEED IN CERTAIN POLYGONACEAE.



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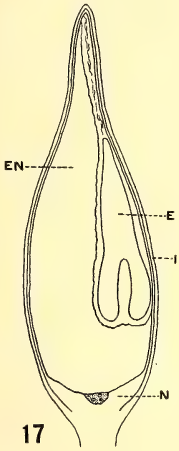

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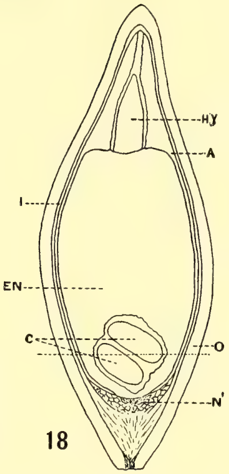

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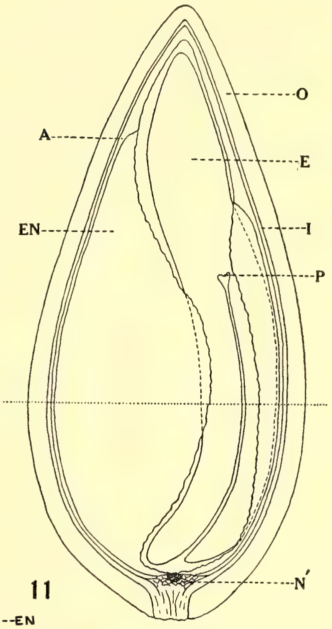

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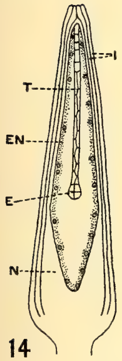

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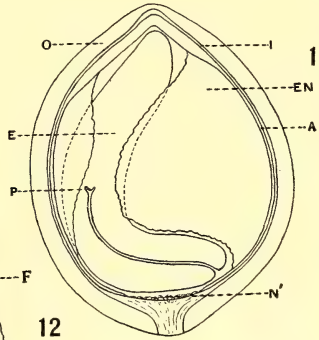

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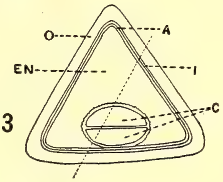

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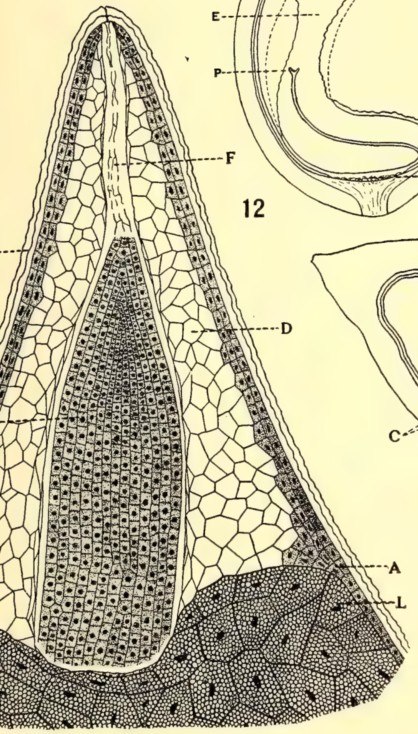

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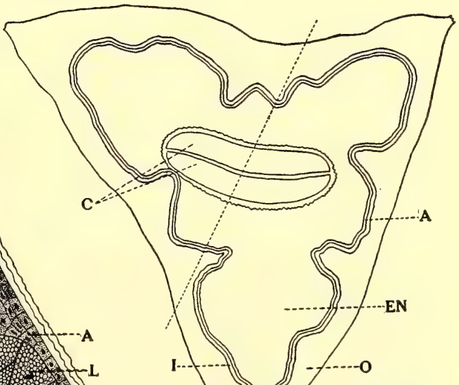

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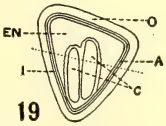

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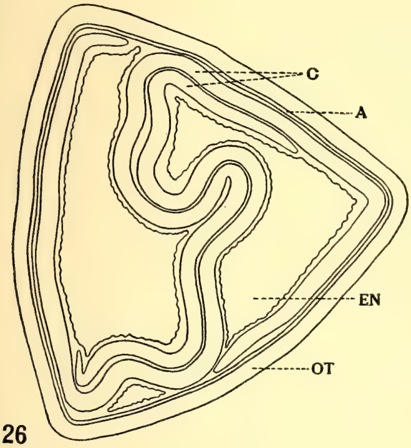
WOODCOCK: SEED IN CERTAIN POLYGONACEAE.



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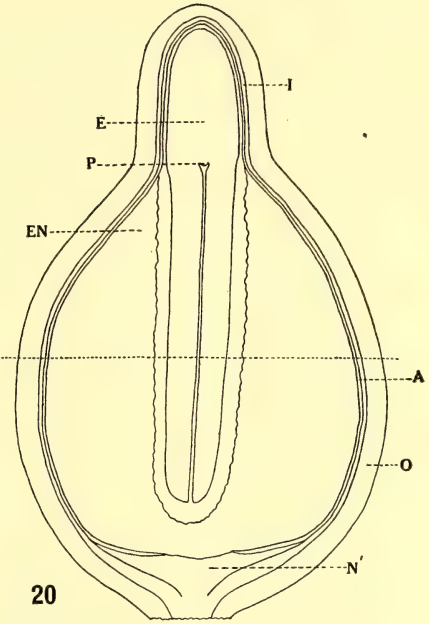

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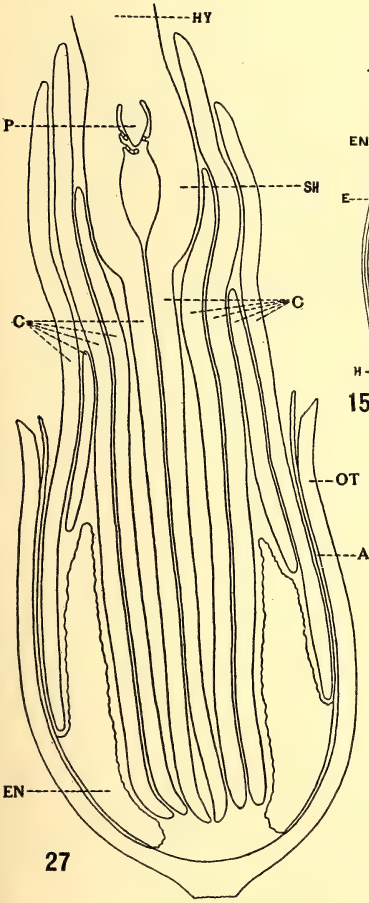
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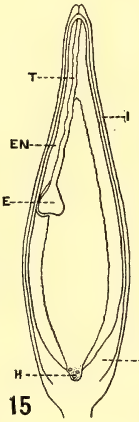

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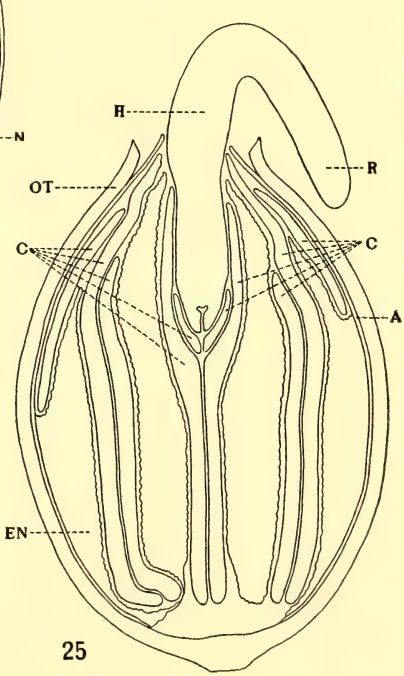

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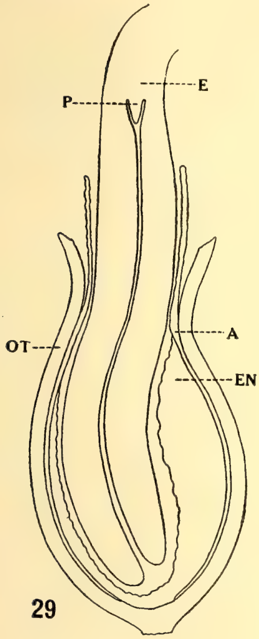

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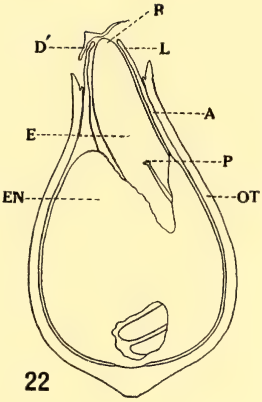
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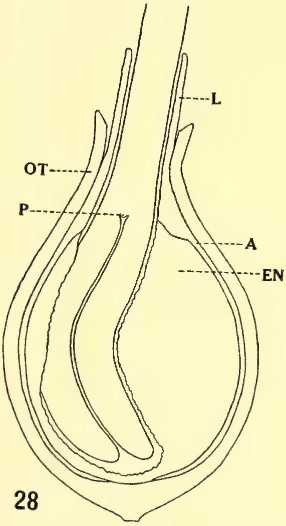

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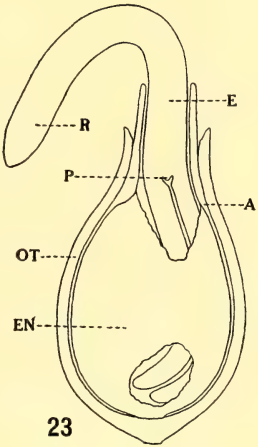

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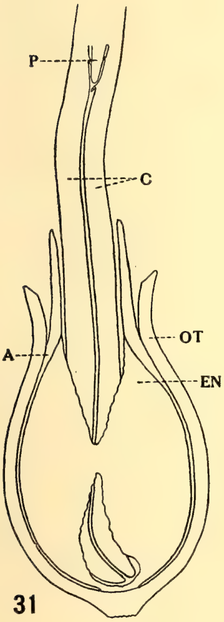

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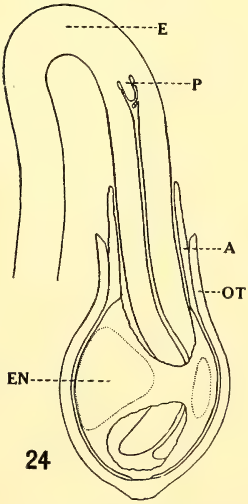

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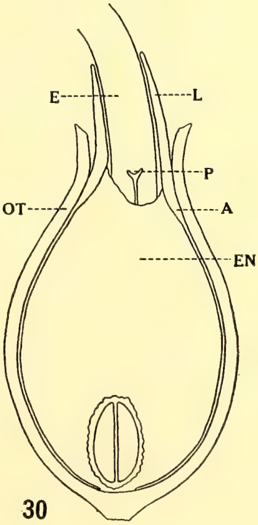

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Woodcock: Seed in Certain Polygonaceae.

SOME ECOLOGICAL ADAPTATIONS OF CERTAIN FERN PROTHALLIA—CAMPTOSORUS RHIZOPHYLLUS LINK., ASPLENIUM PLATYNEURON OAKES.

F. L. Pickett

The part played by the prothallia in the ecological history of ferns has been given but little attention. If the studies on apogamy and related phenomena be left out of consideration the work along this line is very limited indeed. What work has been done may be arranged in three groups, as it has had to do with the increase of growing points by branching of the prothallia or by the production of special proliferations, the influence of light upon the germination of the spore and the development of the prothallium, the influence upon the prothallia of variations of water supply.

(*A*) Goebel (II: 197–210) has summed up the findings concerning the vegetative increase of the prothallia of homosporous ferns. Briefly stated there are two general types of growth, that which leads to the formation of a more or less regular heart-shaped plant, and that which leads regularly to considerably branched forms. Of the second group the *Vittariaceae* and *Hymenophyllum* show a lobular or ribbon-like body which arises by the branching of a simple primary cell plate. In *Trichomanes,* also of the second group, the prothallium is a mass of more or less branched filaments. With reference to those forms which regularly develop heartshaped prothallia Goebel (II: 203) says: "Young fern plants which have not yet formed typical meristem easily pass over again into the filamentous stage in feeble illumination. . . . In older prothallia this only takes place if they have lost their meristem and are enfeebled by unfavorable environment. Commonly these conditions result in the production of pluricellular shoots." To this last group mentioned by Goebel belong the plants often cited as producing adventitious outgrowths following mutilation in hybridization experiments. This author also mentions a case of proliferation by adventitious shoots from the base of an old prothallium of *Osmunda regalis* (loc. cit. I: 49). More recently several authors have found that under peculiar culture conditions the prothallia of many ferns will produce special proliferous buds or gemmae.

In the present paper there will be shown yet another type of vegetative increase, and since the plants under consideration show no tendency to form special proliferous branches or gemmae, the literature dealing with that phase of the subject will be passed with the above brief mention.

(*B*) It has long been held generally that abundant moisture is necessary for the germination of spores and the normal development of the prothallia of homosporous ferns. In fact the ability of any such structures to live without a considerable constant supply of water has but very recently been shown. Goebel in the work referred to above (II: 426) describes special tuberous outgrowths produced by the prothallium of *Anogramme chaerophylla*, which covered with soil, may survive dry seasons and continue growth upon the return of favorable conditions. Little attempt has been made to test the ability of normally developed prothallia to survive extended periods of drought. Campbell (p. 85) cites a case of prothallia of *Gymnogramme triangularis* which were found growing after the dry season in the neighborhood of Stanford, Cal. He also states that the plants of a culture survived exposure to dry air in the laboratory during a whole summer. The results of the first published attempt to determine the extent to which fern prothallia could survive conditions of extreme desiccation appeared in an article by the present writer in 1913 (Pickett, 1913). Discrepancies in the results of that set of experiments, and the possibility of broadening the scope of the work to include other species have led to the present study.

(*C*) The influence of light on the germination of fern spores and the development of prothallia has been the subject of more investigation than both the topics just mentioned. All investigators agree as to the necessity of light for normal complete germination, and to the tendency of plants to develop attenuated or filamentous forms when in reduced light. The present work is concerned primarily with the effect of variation in light intensity under otherwise constant conditions.

This paper embodies the results of attempts to determine the ability of the prothallia of two ferns, grown under control, to survive exposure to conditions of extreme desiccation, to extreme drought conditions as found in nature, and to extremes of temperature as found in nature; to determine the effect of variation of light intensity upon development, and finally by checking up results from controlled cultures with findings in the field, to determine whether or not the peculiarities found are developed or of use in nature.

Camptosorus rhizophyllus Link.

As previously reported (Pickett, 1913, pp. 643–644), prothallia of this fern may survive an exposure of six weeks to air dried by passing through glycerine, but are killed by an exposure of four to six days over sulphuric acid in a closed desiccator. There are two possible explanations of this great variation, either the air is not entirely dried by the passage through the glycerine, or injurious gases are given off by the sulphuric acid. The work of Schröder and of Irmscher on mosses, as well as check experiments performed by the writer, seem to eliminate entirely the possibility of injurious gases being given off by the sulphuric acid. To determine the thoroughness of desiccation the former experiments have been repeated and new apparatus planned to furnish more thoroughly dried air.

In the writer's earlier experiments, as a result of reduced pressure secured by means of an aspirator, a current of air was made to pass through two 20 cm. U-tubes containing glycerine and crumpled filter paper saturated with glycerine, and then through small vials containing the material to be desiccated. In this apparatus air bubbles of from 2–3 cc. required 5–10 sec. in passing through the glycerine in the bottom of the tubes. The air was in further contact with the saturated filter paper about 15 min. In the experiments now to be described five methods of removing the moisture from the air have been employed, designated I, II, III, IV, V. To secure pressure, positive or negative, for the production of a constant stream of air the apparatus shown in text-fig. 1 was used. Water from the tap allowed to flow through *W* into the funnel in a stream about 1/3 as large as the bore of the tube *B* (4 mm.) in passing intermittently through the reverse curve at *B* carries bubbles of air with it into the aspirator jar *C*. The continuous stream of air and water passing into *C* tends to force the air through the tube *V* to *E* and thence through the other pieces of apparatus. Limiting the flow of air by means of screw cocks at *I* causes an increase of pressure in *C* until first the water and then the surplus air is forced out through *D*. The heights of tubes *D* and *B* determine the pressure available, and the number of tubes

(*B*) leading into *C* determines the volume of air available. In the present work $D = 1$ m., $B = 2.5$ m., and two of the latter were used to keep three sets of apparatus running. Tubes of the number

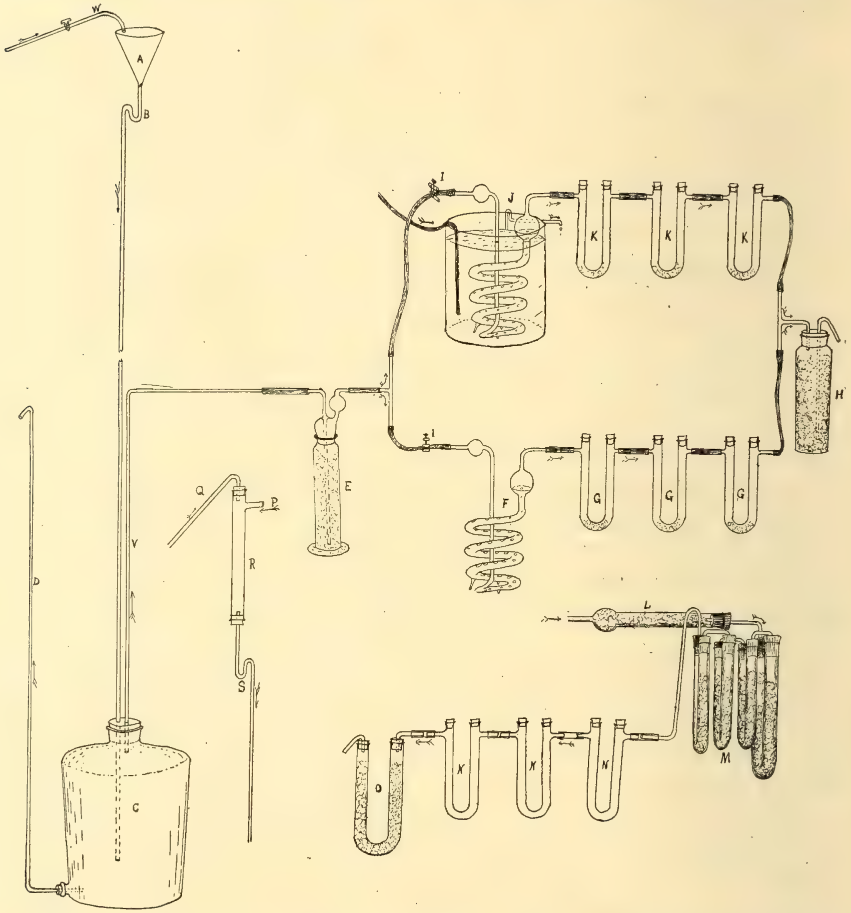


FIG. 1. Desiccation apparatus. *ABCD*, pump for securing positive air-pressure. *W*, inlet from water main. *P–S*, pump to furnish negative pressure. *E*, first drier filled with filter paper saturated with glycerine. *H*, *O*, checks filled with fused calcium chloride. *F–G*, glycerine desiccator and tubes. *I–K*, sulphuric acid desiccator and tubes with cooling jar about Winkler's tube. *L–O*, calcium chloride desiccator and tubes. (× 1/12.)

and dimensions given furnish abundant pressure and volume for easy control. Negative pressure was secured by using the pump *P–S* instead of the funnel tube *A–B* and allowing the water and air to flow from the free end of the tube below. Water passing slowly from the tap through *Q* into the chamber *R* carries air through the curve at *S* and reduces the pressure in *R* so that a current is forced through *P* by external atmospheric pressure. When *P* is connected with the delivery tubes of the other pieces of apparatus the air current passes through them as before. Control of the air current is secured by screw cocks as above. The actual difference of pressure in the desiccating tubes with the different methods of securing pressure is slight, and as results have shown, is wholly negligible for the work in hand.

I. A glycerine desiccator was so arranged (text-fig. 1) that a current of air passed first through a wash bottle, *E*, filled with crumpled filter paper saturated with glycerine, and then as bubbles of approximately .1 cc. capacity, through a column of pure glycerine 1 m. long in a Winkler's potash tube, *F*. The passage of a bubble through this tube required 10–12 min., the time variation being due to variations of temperature and the resulting difference in the viscosity of the glycerine. The dry air next passed through 12 cm. tubulated drying tubes, *G*, and finally out through a 250 cm. wash bottle, *H*, filled with fused calcium chloride. This last element was used to prevent any possible entrance of moisture by diffusion or by currents resulting from changes in temperature. The clumps of plants and soil were carefully removed from the culture saucers when the soil surface began to show dry, and placed in the drying tubes, *G*. They were thus directly in the path of the current of dry air.

II. The second apparatus was an exact duplicate of that described under I, but the Winkler's tube contained a 1 m. column of c.p. sulphuric acid, sp. g. 1.84. The air bubbles, of about .03 cc. capacity, passed through the acid in a little less than 1 min. In check experiments the presence of injurious gases was guarded against by placing the tube, *J*, in a jar, as shown, through which a continual stream of water passed, keeping the temperature of *J* at from 13° to 15° C.

III. A third set of apparatus was so arranged that the current of air passed through a 100 cc. calcium chloride tube, *L*, and then through four U-tubes, *M*, all filled with medium size lumps of fused calcium chloride. This series of tubes was equivalent to a 1 m. column of the chloride. From the tubes the dry air passed over the specimens in the drying tubes, *N*, and out through the final check tube, *O*, filled with the chloride. To insure continual drying of the air the chloride tube, *L*, was so connected that at the beginning of deliquescence of its contents it could be replaced by a fresh tube. A current of carbon dioxide was made to pass through this apparatus for 2 hrs. and then a current of air from the pressure apparatus for 18–24 hrs. before the specimens were placed in the drying tubes. In use the rate of air passage was slightly more rapid than in I.

IV–V. As checks on the experiments in which air currents were used two Fresenius closed desiccators were used, one (IV) with a 2 cm. layer of fused calcium chloride in the bottom and the other (V) filled 1 cm. deep with c.p. sulphuric acid. Parallel check experiments with soil and green tissue show the desiccation in the air currents and in the closed desiccators to be the same. Because of the slow passage of air in I and II, but small quantities of material will be dried quickly.

C. rhizophyllus, Extreme Desiccation.—Portions of soil of about .25 cc. volume, bearing well-developed prothallia with mature antheridia and archegonia were removed from the culture saucers to the drying tube as noted above. Several such clumps of soil and plants were placed in a tube at one time so that portions could be removed each day as wanted, with a minimum danger of mutilating the plants. For recovery the desiccated soil and plants were placed lightly on moist sterile soil under a bell jar in full diffused light in the greenhouse. In all cases recovery or survival has been taken to mean the ability to continue growth or to produce living sperms. The averages of results of experiments with the various methods are given below. The time in days is reckoned from the time when plants were put into the drying tube up to that of removing a clump which failed to show a living plant. The variation did not exceed one day in any case of duplicate experiments. The clumps were removed for revival at 8 A. M. each day. No attempt was made to determine the exact number of hours required for fatal exposure. Individual differences of plants would make such work extremely difficult; and the results would in the end be but averages for the species, such as we have by the method employed.

Results.—	I.	Glycerine desiccator	all plants dead on the	7th day.
	II.	Sulphuric acid desiccator	“ “ “ “ “	7th “
	III.	Calcium chloride tube	“ “ “ “ “	6th “
	IV.	Fresenius, calcium chloride	“ “ “ “ “	6th “
	V.	Fresenius, sulphuric acid	“ “ “ “ “	6th “

These results agree very closely with those secured with the closed desiccators as formerly reported (Pickett, '13), the slight discrepancy being accounted for by the use of larger and older plants in the present work. They also clear up the discrepancy between the results with the glycerine desiccator and the sulphuric acid desiccator as previously reported, and give an important check to the value of the three agents used in drying currents of air. While not quite as striking as earlier results, in view of the extreme conditions, they are worthy of consideration. This becomes more evident if the data be compared with the following from non-resistant forms. Prothallia of *Onoclea struthiopteris*, *Dryopteris stipularis* and *D. mollis* used in check experiments have been killed by allowing soil of cultures to be dry for 2 days in sunlight, and can withstand but 2–3 hours exposure in the desiccator.

It may well be noted here that several times small plants, bearing 10–15 leaves, of *Polytrichum piliferum* were placed in the drying tube with the fern prothallia and recovered completely although left 3–5 days after the prothallia were dead. Small plants of an unknown *Bryum* survived similar exposure without damage. This is taken to show that injurious gases were not present in harmful quantities, if at all.

Cultures for experimental work were regularly made by sowing free spores and crushed sporangia on rich loam in 12 cm. unglazed clay saucers with perforated bottoms. These saucers were placed in others 18 cm. in diameter. Irrigation was controlled by supplying water as needed to the outer saucer. Both saucers and soil were sterilized 1.5–2 hrs. at a temperature of 130° C. in an autoclave each day for three days before the spores were sown. The cultures were covered with tall bell jars with one side raised by a cork 1 cm. high to provide ventilation. As has been already reported (Pickett, 1914) cultures of this fern do not show uniform development. Some spores germinate in ten days or less, and by rapid growth produce sexually mature plants in ten to twelve weeks. Other plants develop slowly, and some spores lie dormant for three to four months under conditions suitable for germination. Numerous cultures have been made from spores of fronds collected in the field in March and April, a clear proof that some spores even live through the winter.

Extreme Natural Conditions, Experimental.—Cultures of mature plants were exposed to long dry periods in full sunlight and in full diffused light in the greenhouse, the conditions being approximately those found in nature in the growing season. Diffused light was secured by means of a muslin screen 2 m. above the culture tables or by means of a double layer of filter paper pasted to the bell jars and half covering them. As already found, the exposure of air-dry cultures to full sunlight during periods of three to five weeks proves fatal. Cultures exposed for nine weeks to normal dry air without water but shielded from the direct sunlight showed a few living plants. One of the cultures has been subjected to periods of drought one to two weeks in length during a total period of sixteen months, and has but rarely been watered twice in succession without an intervening dry period. Occasionally it has been flooded, and has shown a new crop of sporophytes after each flooding. 25 per cent of the plants of this culture were living at the end of the sixteen months period, although they had undergone changes, as will be noted later.

On December 8, 1915, soil cultures with mature prothallia were placed outside the greenhouse, protected from direct sunlight by the building and an adjoining wall, and covered by bell jars. On March 26, 1914, cultures returned to the greenhouse showed all plants dead or injured. Injured plants had much the appearance of plants injured by too long exposure to drought, showing old dead tissue and groups of younger active marginal or meristematic cells. The lowest temperature to which these cultures were exposed was − 12° C. in this protected location, although temperatures around − 6° C. prevailed through January and February.

Field Notes.—A brief review of the weather conditions during the summer of 1913 will give the field notes their full value. The summer was extremely dry and warm in southern Indiana. Following the flood period of March 23–27 with 9.2 in. of rain, April was much drier than usual. Then a period of 146 days, from April 30 to September 12, showed but 35 showery days, with a total precipitation of but 6.3 in. Between Sept. 13 and 30 there was a rainfall of 2.78 in., and then the drought continued up to Oct. 17, with but .48 in. in the interim. During the same period the following temperature conditions were recorded:

	Mean Maximum	Maximum
May	25.5° C.	35.° C.
June	28.2	40.
July	34.4	41.9
August	33.2	38.3
Sept. 1–12	35.	38.8

This is the official record for the U. S. Weather Bureau station at Bloomington, Ind., and the figures show the readings of standard sheltered instruments. When placed in direct sunlight without reflecting surfaces and in freely moving air, the instruments showed a maximum of 54.4° C. These conditions were disastrous for many forms of vegetation. Midsummer and late annuals were killed outright. Ferns, except the early fruiting species or those growing in most favorable localities, failed to mature spores. Even the hardier soil-growing mosses showed remarkable mortality.

Camptosorus patches were visited in October and December, 1913, and in early March and April, 1914. In more exposed places, on limestone ledges, but few spores were matured in 1913, and most of the sporophytes suffered, some being killed by the dry summer. No prothallia were found in these places up to April, 1914, although they may usually be found in the autumn. On April 16, careful search was made in a wide wooded ravine where *Camptosorus* is abundant on large blocks and fragments of limestone which are matted with mosses, especially *Anomodon attenuatus* and *Brachythecium oxycladon.* Because of the numerous springs in this ravine, the drought was not as severe as in more exposed locations. Almost a full crop of spores had been produced in 1913, and on the date given, a few prothallia were found on soil in pockets protected by the mat of moss. Some of these prothallia showed sporophytes. A few were quite large and showed marginal outgrowths like those found on old prothallia of cultures, to be described later. Of course all these prothallia must have survived the winter, as might be expected with the protection of such a location. The largest plants were very much like those of sixteen months old greenhouse cultures, and very probably had endured the tempered drought of the summer of 1913. Fronds collected at this place on March 31 have furnished an abundance of readily germinating spores. Usually prothallia are easily found about colonies of *Camptosorus* from late August to cold weather, and again after April, although they do not survive winter in exposed situations in this region. It would be of great interest to the writer to know how far north they have been found regularly surviving the winter.

Proliferation or branching of fern prothallia is by no means unknown. Some forms produce normally a branched protonema-like form. Some have been described as producing hair-like outgrowths from marginal cells under conditions of special stimulus. Other forms produce proliferations—sometimes mistaken for apogamous sporophytes—from unusually active cells of the surface. As described by Goebel (I: 49), prothallia of *Osmunda regalis* when old may produce branches from the older meristematic region. The outgrowths of *Camptosorus* do not rightly belong to either of these groups. They were first described briefly by the writer in the Botanical Gazette of March, 1914. In old prothallia which have been subjected to long periods of drought, the older portions die away leaving groups of living marginal cells, or limited living areas below the margin. The older portions after they are no longer vegetatively active, conduct water to the active cell groups for a time. Some of the outgrowths are small, irregular, without apical cell or group and are but one cell in thickness. These bear rhizoids, and an abundance of antheridia but no archegonia. Others soon show apical groups, develop typical prothallial forms and produce rhizoids, meristematic cushions and both antheridia and archegonia. In short, these marginal proliferations show all the characteristics of normal prothallia, and after a time, through the breaking down of the tissue of the original plate, become entirely independent. Their sex organs are normal, and they produce sporophytes abundantly, sometimes while yet attached to the old prothallial mass. While they are found most abundantly on plants which have been subjected to unfavorable conditions, their formation is not wholly dependent upon such conditions, as evidenced by the fact that on some plants the marginal activity has proceeded far enough for independent growth by the time the first archegonia have died. The number of proliferations varies. Usually but two or three reach any considerable size, but in other cases as high as fifteen such independent growths have been counted. Fig. 38 shows a plant with rather unusual marginal development. In this figure *Q* shows the oldest portion of archegonial meristem, from which yet older dead tissue has broken away. In the irregular growth of the plant this cushion has taken a curved form, and its latest development is at *O* where a sporophyte has appeared. At points *A* to *R* marginal growth has produced proliferations. *P* was attached at point *T* and broken away in preparing the specimen for photographing. The dark central area and much of the lighter area around it are dead tissue. The root of a young sporophyte is shown at *S* and the leaf and stem buds at *O*. The first leaf was broken off in mounting. The mottled mass just behind the sporophyte at *O* shows the region of numerous antheridia and archegonia. It must be borne in mind that these proliferations result from the activity of *groups* of marginal cells and not from the activity of single cells as in the formation of proliferous buds or gemmae, and that they do not appear as the result of mechanical injury to the parts producing them.

Summary of Camptosorus rhizophyllus.—The following freatues of the prothallial life of *Camptosorus* indicate to what extent the sexual generation of this fern is a factor in its distribution: Mature prothallia withstand practically unlimited interrupted drought as found in nature, if not exposed to direct sunlight, producing sporophytes through fertilization at the time of occasional showers.

The prothallia may survive exposure to experimental conditions of extreme desiccation for periods of four to six days.

A temperature of – 12° C. is fatal to most exposed plants.

Where winter exposure does not result fatally, the direct production of marginal proliferations may continue the prothallial life indefinitely.

The following facts concerning spore production and germination are also important factors: Spores are shed through a long period of time, some being retained in the sporangia until the spring following their formation, thus making possible sexual multiplication where the winters are too severe for the survival of the prothallia.

The spores germinate irregularly in point of time, thus offsetting the rather greater susceptibility of young prothallia to damage by drought.

ASPLENIUM PLATYNEURON Oakes.

In southern Indiana *Asplenium platyneuron* Oakes, is very common, growing at times on the sides of damp wooded ravines, where fronds 30–40 cm. in length are not rare, and occasionally in greatly reduced form on dry limestone cliffs and ledges. It is most abundant, however, in open woods along high ridges and on dry hillsides. The presence of many juvenile plants in the last named habitat has suggested the likelihood of conditions favorable for gametophyte development, and thus led to the present study. Most of the material for this work was taken from a steep slope of clay and light humus overlying limestone, with a southeast exposure. No springs are in evidence at this place and the hillside is but slightly covered with briers and bushes grown up since the timber was cut off three or four years ago.

In general the problem and means of attack are the same as in the case of *Camptosorus*. The same culture methods have been used, and the same apparatus for securing extreme conditions. In fact cultures have been kept along beside those of *C. rhizophyllus*, and in the desiccation experiments tubes containing the two plants were attached in series so that they received exactly the same exposure. The important differences in treatment were in the experiments involving varying light conditions.

Fronds were collected June 24 and October 18, 1913. The latter collection was of fronds grown up after the rain of September 12. Cultures were prepared on soil, also on culture solutions as described in a later paragraph. The following features in the germination and development may be stated as generally true. The growth of the prothallia is much more uniform than in the case of *C. rhizophyllus*, although on soil cultures it is not unusual to find germinating spores and plants with twenty-celled plates side by side. The prothallia grow rapidly and symmetrically in either sunlight or full diffuse light. In twenty days after the spores are sown plants of five to fifteen cells may be found. At the end of ten weeks most of the plants show mature antheridia and archegonia. These points are true for cultures grown at an average temperature of 21° C. Only after the plants were sexually mature have they been subjected to the various conditions of experiment touching resistance to desiccation and low temperature.

Extreme Desiccation.—Plants were exposed to dry air in the desiccating apparatus with the following results:

I. Glycerine desiccator	all dead after	6–7 days.
II. Sulphuric acid tube	" " "	6–7 "
III. Calcium chloride tube	" " "	5–6 "
IV. Fresenius-calcium chloride	" " "	5–6 "
V. Fresenius-sulphuric acid	" " "	5–6 "

The variable time of 5–6 and 6–7 days in these items is so placed because quite regularly a few plants of average size and normal appearance, have been found living after the shorter exposure. The writer is inclined to consider this a demonstration of remarkable individual variation in this respect comparable to that shown by the prothallia of this plant to a very unusual degree in other ways, to be noted later.

Extreme Natural Conditions.—Cultures allowed to become dry in the greenhouse showed no damage after three weeks, in diffuse light.

As an extreme trial a culture was watered October 8, 1913, and placed in full diffuse light with a temperature range of 12°–21° C., in air not in contact with water surface or sprays. On October 10 the soil surface appeared dry. Small portions were removed for revival at intervals until March 28, 1914. At that time, after 171 days without water, 20–25 per cent. of the plants were in condition for growth.

Cultures allowed to become air dry in sunlight at a temperature range of 13–25° C. during January, February and March, showed about 50 per cent. of the plants living at the end of four weeks, and showed a few living plants after six weeks of such exposure. Weather conditions make great variations in the results of these experiments, and the above data must be taken as the average of many repeated experiments. It has been very interesting to watch cultures of *A. platyneuron* and *C. rhizophyllus* exposed to the same light conditions. The greater resistance of the former to combined light and drought is very noticeable.

Winter Conditions.—Cultures placed outside on December 8, 1913, showed every plant living when brought inside on March 26, 1914. The lowest temperature to which these were exposed was − 12° C. and this seemed not to injure the plants in the least. This result was true with both dry and flooded cultures. Experiments to determine the minimum temperature limit have not been arranged, but field observations have indicated that a temperature of − 23° C. is not necessarily fatal.

Reaction to Special Light Conditions.—It has been stated above that the prothallia of *A. platyneuron* grow well in direct sunlight, other conditions being favorable, and that they are less liable to injury than those of *C. rhizophyllus* when cultures become dry in full light. It is now to be shown that they have a marked tendency to modify their growth as a result of variation in light intensity.

Spores and crushed sporangia were sown on a modified Knop's solution in 6 cm. petri dishes and stender dishes. Check cultures were made on distilled water and sterilized tap water. The Knop's solution was prepared as follows: A. KNO_3, 2 g.; $MgSO_4$, 2 g.; K_2HPO_4, 2 g.; Aq. dist., 2,000 cc. *B.* $Ca(NO_3)_2$, 4 g.; Aq. dist., 3,000 cc.

For use one part of *A* is added to three parts of *B* and boiled fifteen minutes. A series of check cultures was made with a solution similar to the above but with sterilized rain water instead of distilled water. No difference could be noted between the two series of cultures.

Variations of temperature between 13° and 25° C. affect development only as to rapidity. Cultures placed where they received equal light, but with a constant difference of 11° C. in temperature, develop similarly in every way, but those in the warmer place grow much more rapidly. This is true of plants in all culture media. Plants of cultures changed from a temperature of 13° C. to 24° C. show none of the peculiarities described below, as long as the light conditions remain the same.

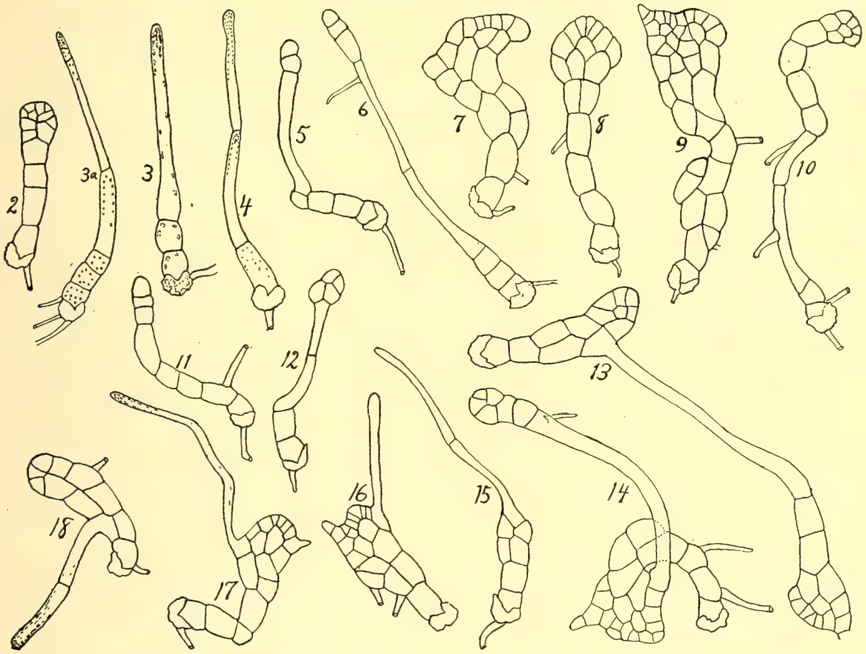
Spores may begin germination and produce one or two rhizoids when sown on distilled water, but will only very rarely show any cell division or chlorophyll formation. One series of such cultures was started August 30, 1913. After being subjected to a temperature of 16° C. and diffused light (.083) for seven weeks these cultures showed more than 90 per cent. of the spores with one or two rhizoids, varying in length from five to fifty times the greater spore diameter. At the end of this period nutrient solution was added and all the spores with rhizoids continued normal germination. Other cultures were allowed to wait ten to twelve weeks before receiving nutrient solution. Only about 50 per cent of the spores produced plants after the twelve week period.

Prothallia submerged in the culture solution tend to develop long protonemal chains of cells (fig. 20) and but rarely produce plates. The growth of submerged plants is also much slower than that of floating plants. Plants on the surface of the culture fluid develop in every way as plants on soil, but do not form antheridia or archegonia. At least, cultures kept under favorable conditions for eight to nine months show no sex organs. In this discussion floating plants are considered except where otherwise stated.

In the attempt to determine the influence of light of different intensities and of varying intensity, cultures with prothallia at various stages of development have been used. As a basis for comparison the history of one culture is here transcribed in detail from the record. The light values given were determined by means of a Solio photometer, and are based on 1. = full sun 1.00 P. M. of a clear day. Spores were sown on Knop's solution in a stender dish, October 13, 1913, and left in the greenhouse vestibule at an average temperature of 21° C. and with a maximum daily light value of .083.

October 28, nearly all the spores have germinated and most of the plants are composed of three to five plump cells in chains, with abundant chloroplasts. *Culture was placed under a saucer raised 1 cm. from the table, light value .011.*

November 4, culture shows chiefly 4–10 celled prothallia. Some plants show plates of 2–6 cells. Some distal cells are attenuated with the chloroplasts crowded in the extreme end (figs. 4, 23). For position of chloroplasts see also figs. 26*b*, 32, 33.



FIGS. 2–18. Camera lucida drawings of prothallia of *A. platyneuron* grown on Knop's solution. (× 240.) See text for description.

November 11. Plants show growth along three lines, increase in number of cells in plates or chains and increase in length of attenuated cells. Largest plants show 20 cells. *Culture removed to light of 0.8.*

November 17. Plants show rapid growth of plates with the beginning of apical groups. Attenuated filaments show normal plump cells at their distal ends, and in some cases, show divisions preparatory to plate formation (text-figs. 5, 6, 11, 12).

November 25. Plants show continued growth as last noted. Some show plates up to 40 cells where no attenuation had taken place, and up to 20 cells at the tips of attenuated cells. Some filamentous forms show branches (text-figs. 7, 8, 9, 10).

December 1. Continued growth as above. *Culture returned to reduced light, .011.*

December 17. Branches have grown from tips and sides, and rarely from apical cells, in every way like the attenuated growths referred to above (figs. 15, 16, 17, 18, 29, 32, 33).

December 29. The extensions have grown up to a maximum of three cells in length (figs. 25, 26). *Culture returned to strong light, 0.8.*

January 5. Plants show normal beginnings of plates at the tips of second attenuations (figs. 13, 14, 19, 21, 22).

This culture was allowed to remain in full light. Each plate formed at the tip of an attenuated cell grew normally as an independent plant. The primary plates also continued normal growth. In each citation given above the figures are from plants taken from one culture at one time, and usually from a single mass removed on a needle point.

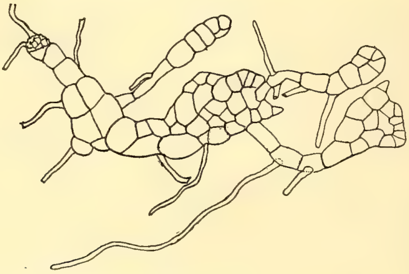


FIG. 19. Prothallium of *A. platyneuron* showing outgrowths from different parts to form independent plants, as a result of a period of reduced light followed by a period of stronger light. (× 52.) Traced from a photomicrograph.

The above account is that of a typical culture. There were variations in time and extent of reaction, due to weather conditions; but there was always a marked reaction in *some plants* and a seeming indifference *in others*. This individual difference is a point of special interest, manifested at every change in condition, and will be discussed at length.

Every culture subjected to variations of light intensity sufficient to produce changes in the manner of growth, has presented upon examination two groups of plants. The greater number of plants show reactions as described above. Some plants—about 25 per cent of each culture—on the other hand, show no noticeable reaction to light changes. In order to make sure that age had nothing to do with such variation, cultures were made by sowing spores on distilled water, and after three weeks when all viable spores had produced rhizoids, adding Knop's solution. The resulting growth was very uniform in rate (fig. 24), and most of the plants showed the first division indicative of plate formation at the same time. But when such a culture was removed from full to reduced light, the results were by no means uniform. The larger number of plants show the formation of attenuated cells already described. But side by side with these forms are other plants showing no tendency toward such growth. Again the same situation is found in cultures subjected to two to four alternating changes as in fig. 31 *a–h*. Here some plants are shown continuing regular growth and plate formation without any sign of branching or attenuation. The growth, measured by the number of cells formed, is not quite as rapid under conditions of reduced light as in full light. It must be noted, however, that in plants grown from the first in light just too weak (.011) for normal development this difference does not appear (fig. 30). The sensitiveness to light variations is also shown by the following data. Cultures were grown continuously in light of maximum value of .025 and produced plants six to ten cells in length, slightly attenuated. From the middle of December up to the latter part of February there were alternating periods of three to ten days of very bright and very dark weather. During this time the cultures mentioned above showed the zones of attenuated cells and plates, just as produced by the more extreme changes of the experiments (fig. 27). This at least suggests that the changes in growth are due to the *variation* in light intensity and not to its absolute value. This view is further substantiated by the fact that in a few cases plants grown in light of maximum value of .025 and then brought into light of .8 maximum value produced the outgrowths regularly produced after a change from strong light to weaker light. It seems from this that the variation in light intensity is only a stimulus to a change in growth, and does not determine the nature of that change. Finally it must be noted that as a result of changes of light intensity some plants tend to branch rather than produce attenuated cells. Extreme cases are much like that in fig. 20. A younger and more moderate case is shown in fig. 28. Such plants finally produce several apical groups and plates (fig. 34).

As has already been suggested there is much difference in the origin of the branches and attenuations which follow the change in light intensity. In a small number of cases the apical cell produces the outgrowth (figs. 25, 26*a*, 32). But more often such growth does not take place from the apical cell or any of the neighboring cells, but from older cells in regions of reduced activity (figs. 16, 17, 18, 19, 21, 22, 26*b*, 29). When plants with plates 1–2 mm. wide are subjected to reduced light the branches or attenuated cells are produced by any part of the margin, from the original protonemal cells up to the apical group, and even at times by included cells growing out perpendicular to the surface of the plate. In other cases the whole apical group is pushed out by a band of slightly elongated cells until a distinct pluricellular branch is formed (fig. 35). To make sure that these results were not unduly influenced by the fact that the plants were grown on culture fluids, regular soil cultures were subjected to the same conditions. The results were the same with the exception that, in the case of plants with well developed plates .5 mm. or more in width, the production of broad outgrowths like fig. 35 was rather more usual than with the plants of water cultures.

Summary of Reaction to Light.—The results of experimental work relative to reaction to light stimulus may be summed up as follows: Plants develop normally in almost identical manner under approximately constant light intensities within a wide range.

Most of the plants respond to a sharp reduction of light by producing elongated cells or pluricellular branches.

Some plants show a similar reaction when the light intensity is increased.

Some plants do not respond to variations in light intensity by any peculiarity of growth.

It seems that the *change* in light intensity stimulates unusual growth in most of the plants, but the plants show a marked individual variation in their response to that stimulus.

Field Work.—The extreme conditions of the summer of 1913 have already been given. To show that the *Asplenium platyneuron* fields under observation received the full force of these conditions it need only be said that many clumps of mature sporophytes were killed outright, after maturing a few spores in the early summer. On December 3, 1913, a careful search was made for prothallia in the field. Numerous well developed plants were found on the soil, limestone fragments and dead twigs or leaves about the old sporophyte clumps. Most of these ranged from ten to fifteen cells up to those maturing their first antheridia, and averaged a little smaller than the plants of greenhouse cultures from spores sown September 4, 1913. A second group of prothallia averaged 2–4 mm. wide and showed earlier dead tissue and antheridia and archegonia long past mature (figs. 36, 37). The younger portions of these plants bore mature sex organs and in a few cases sporophytes of one or two leaves. The similarity of the younger plants in size and development to those in cultures from spores collected and sown September 4, just before the rain of September 12 first made possible germination and growth in the field, leaves little room for doubting that these plants were produced by the germination of spores in the fall of 1913. The plants of the second group were much larger than those of cultures started August 30. In fact they were quite similar in every way to plants examined in April, 1914, of cultures started in September 1913 and allowed to remain out of doors during most of the winter of 1913–14. Their age is evidenced by the dead basal areas. There are two possible explanations of the appearance of these plants. The first, that the plants grew from spores which had lain dormant through the winter and had germinated in the early spring of 1913, need hardly be considered, because no indications of a tendency to lie dormant has been found in experimental cultures, and because the growing season in the spring of 1913 was too brief for the production of plants of the size found. The second possibility, and what seems to the writer the only plausible explanation, is that the larger plants were produced by the germination of spores in the autumn of 1912. This view includes the belief that the plants had survived the winter of 1912–13 with a minimum temperature of − 23° C. in the field. The experimental data presented above have prepared the way for such a belief, and the finding of many living plants in late January, 1914, and even in April, makes the possibility of surviving the winter not only a belief but a certainty. The writer feels quite safe in saying that a considerable portion of the prothallia of this fern, grown from spores germinating in the late summer and autumn, live through the winter and produce sporophytes the next summer.

The following facts touching the reaction to light have been noted in the field. The usual place of growth of prothallia is under old leaves in crevices in limestone or in depressions in the soil. Any chance breeze may by shifting the covering produce variations in light intensity equal to the widest range in the writer's experiments. In fact nearly all the facts of behavior noted in the experimental cultures have been verified in the field by the finding of forms from the branching protonemal type up to independent secondary prothallia produced by outgrowths from old plants to which in some cases they were yet attached. That specimens found in the field are *A. platyneuron* cannot be determined with absolute certainty. The absence of other ferns in the immediate neighborhood and the abundance of prothallia close around old clumps of sporophytes leaves little doubt on this question. Careful comparison of plants from the field with those of cultures, including measurements of cells, have been made, however, and in some cases sporophytes large enough to be recognized as juvenile forms of *A. platyneuron* have made the identification more certain.

Summary of Asplenium platyneuron.—The facts of the growth and development of the prothallia of *A. platyneuron* of ecological importance may be summed up as follows: There is a variation of a few weeks in the time required for the germination of spores.

The prothallia in experimental cultures withstand a temperature of – 12° C. without injury, and in the field they have survived exposure to a temperature of – 23° C. and later produced sporophytes.

The resistance to extreme desiccation, brought about artificially, is slightly lower than in the case of *Camptosorus rhizophyllus*, but the resistance to extreme natural drought is much more marked than in that fern.

Mature prothallia are uninjured by exposure to repeated periods of three to four weeks of drought as met with in nature.

Extreme sensitiveness to changes in light intensity leads to the production of protonemal branched prothallia, or attenuated outgrowths, either of which may lead to a vegetative increase of the gametophyte and the formation of independent plants.

The prothallia show marked individual differences in reaction to light changes and in the power to survive exposure to conditions of extreme desiccation.

The author wishes to acknowledge his indebtedness to Prof. D. M. Mottier and Dr. F. M. Andrews, of the Botany Department of Indiana University, for their kind encouragement and valuable direction and help in the present work.

Botanical Laboratory,
Indiana University

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DESCRIPTIONS OF PLATES XLIX AND L

PLATE XLIX

With the exception of figs. 27 and 34 these photomicrographs were made with Zeiss 16 mm. apo. obj. and No. 4 comp. oc. The others were made with Leitz No. 1 achromatic obj. and oc. No. 2. Cramer's "slow Iso" plates were used.

Asplenium platyneuron prothallia

FIGS. 20–22. Plants grown in full light, subjected to reduced light for sixteen days and then returned to full light. (× 75.)

FIG. 23. Young plants subjected to reduced light. See fig. 3, 3*a*, 4. (× 75.)

FIG. 24. Group of plants from culture of spores allowed to remain three weeks on distilled water in good light before Knop's solution was added. Photo taken ten days after the addition of nutrient solution. (× 75.)

FIGS. 25, 26. Plants after a second change to reduced light. (× 75.)

FIG. 27. Plants from a culture grown in weak light, showing zones of varying growth corresponding to periods of bright and dark weather. (× 40.)

FIG. 28. Branched protonemal form. (× 75.)

FIG. 29. Plants subjected to a second period of reduced light. This with 21, 22, and 26*b* shows attenuated outgrowths from older portions. (× 75.)

FIG. 30. Plants showing uniform growth in light just too weak for normal development. (× 75.)

FIG. 31 *a–h.* A group of plants from one culture, showing the great difference in response to variations of light intensity. (× 75.)

FIGS. 32, 33. Plants grown in full light and then placed in reduced light, showing attenuated branches from cells near the apical group. (× 150 and 75.)

FIG. 34. Final formation of several plates by a branched form. Two other plates were attached at *a* and *b* but were torn away in mounting. (× 22.)

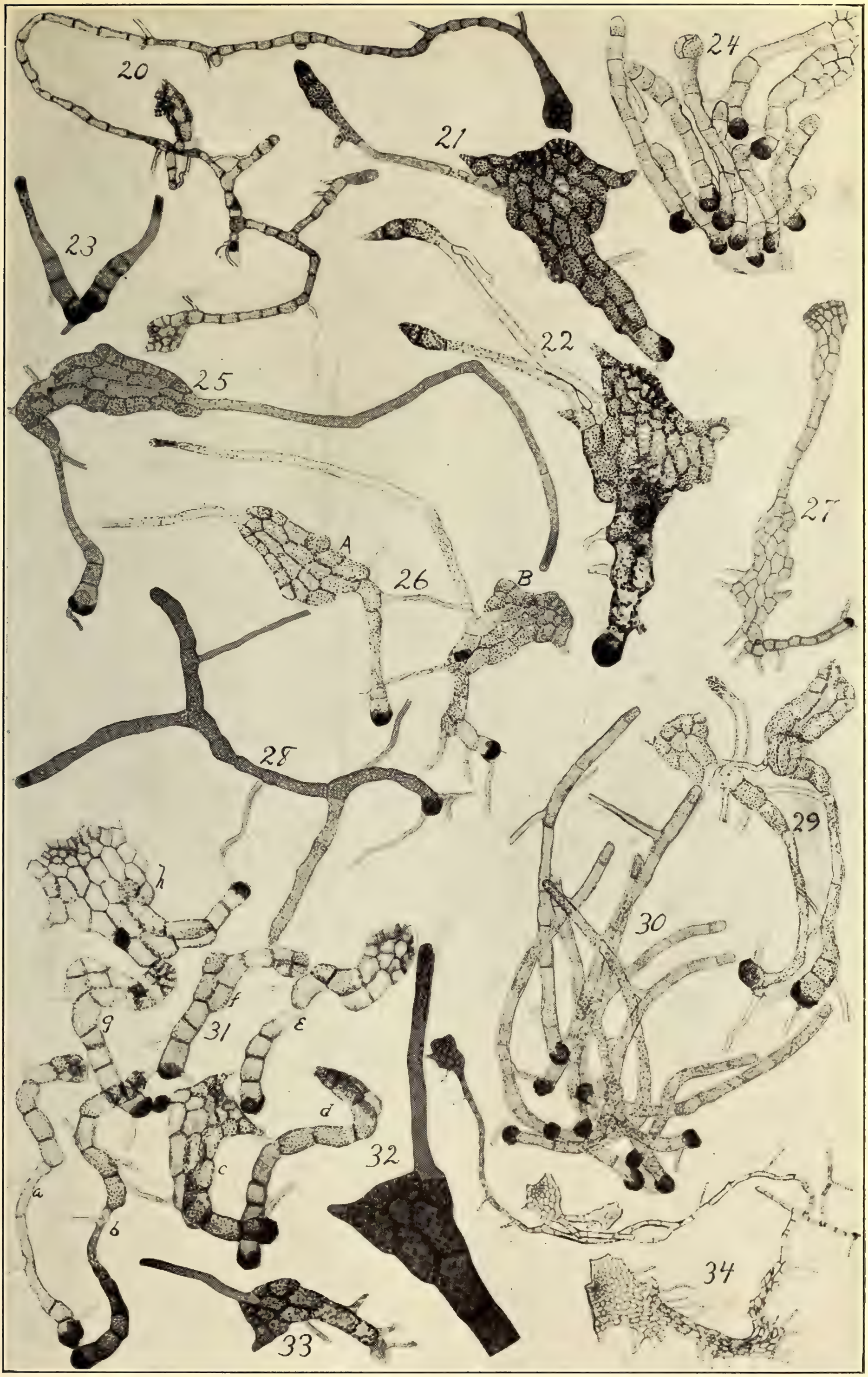
PLATE L

Photomicrographs made with Leitz achromatic obj. No. 1 and oc. No. 2.

FIG. 35. *Asplenium platyneuron.* A plant showing a pluricellular branch, a common form among mature plants of soil cultures changed from strong to reduced light. (× 52.)

FIGS. 36, 37. *A. platyneuron.* Plants collected in the field, probably more than twelve months old. Much of the old tissue was torn away in cleaning and mounting the specimens. (× 52.)

FIG. 38. *Camptosorus rhizophyllus.* Plant from a culture thirteen months old. *A–R*, regions of marginal growth. *P*, an independent secondary prothallium, formerly attached at *T*. *S*, root of a young sporophyte. *Q*, oldest portion of the archegonial cushion. See text for full description. (× 22.5.)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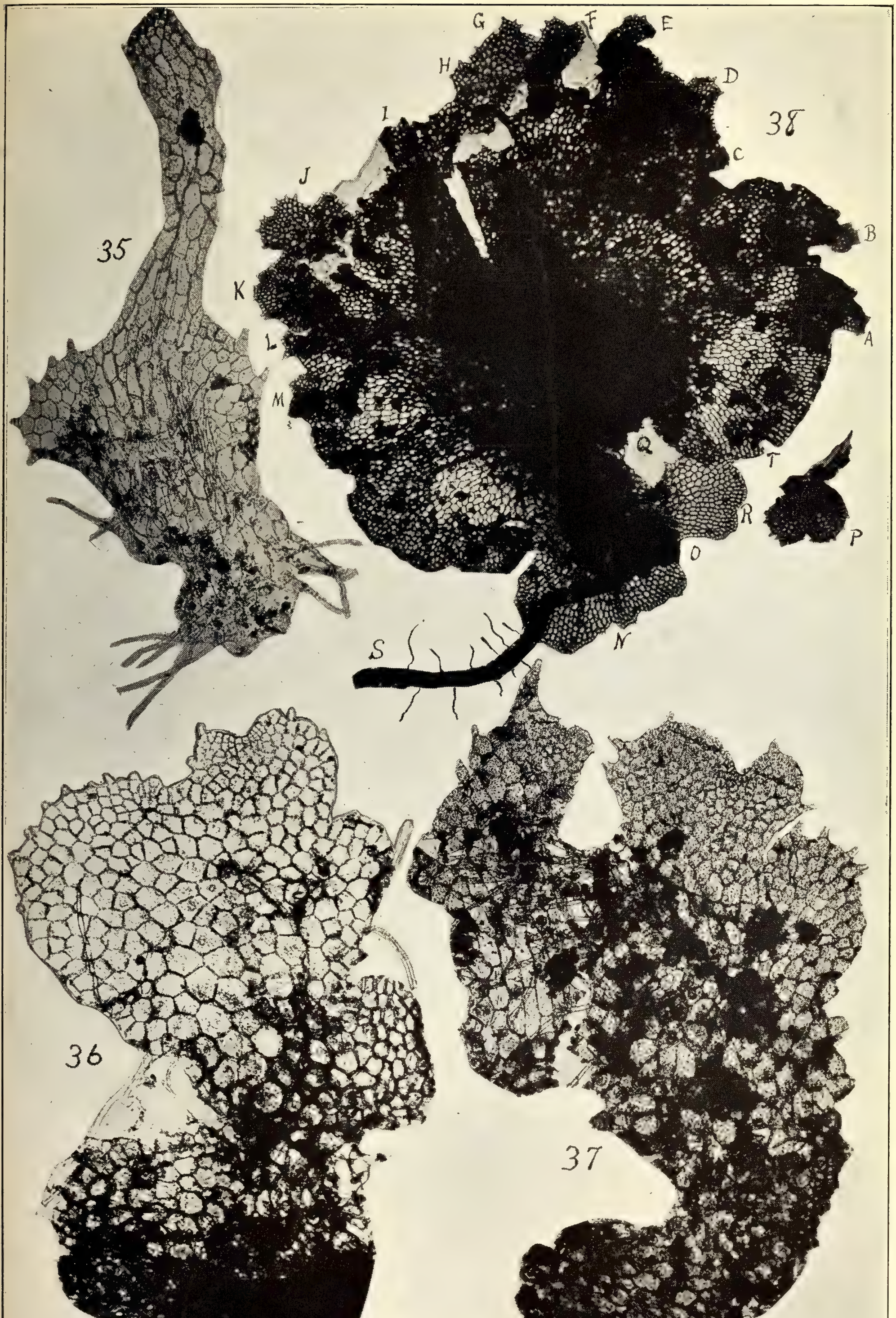
PICKETT: PROTHALLIA OF CAMPTOSORUS AND ASPLENIUM.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Pickett: Prothallia of Camptosorus and Asplenium.

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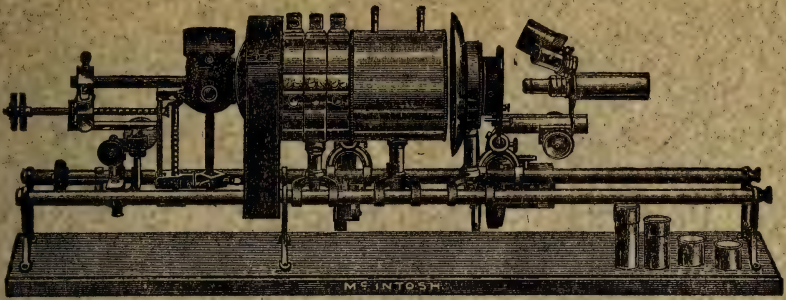

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Anthoceros, sporophyte
Fern, sorus with sporangia
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Vol. I DECEMBER, 1914 No. 10

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THE EXPULSION OF ASCOSPORES FROM THE PERITHECIA OF THE CHESTNUT BLIGHT FUNGUS, ENDOTHIA PARASITICA (MURR.) AND.

F. D. Heald and R. C. Walton

Introduction

General historical consideration of ascospore expulsion from perithecia.—The scope of the present article will not permit an exhaustive treatment of the subject of spore expulsion, but it seems desirable to point out at the beginning some of the more noteworthy observations, and especially those which apply to pyrenomycetous forms.

Forcible expulsion of spores as a means of aiding in their dissemination takes place in many fungi and is accomplished in a variety of ways. The projection of the entire sporangia as in *Pilobolus crystallinus*, or of the single spores as in species of Empusa and Entomophthora, are well known illustrations. Cases of forcible expulsion of spores by Ascomycetes have long been known, and more recently similar phenomena have been demonstrated for Hymenomycetes (4, 7) and Uredinales (5, 7). Although the height of projection is never great it is incomparably greater in the ascomycetes (few mm. to 15 cm.) than in the basidiomycetes, as has been pointed out by Falck (8).

Directing our attention to Ascomycetes, the simultaneous ejection of the spores from a considerable number of asci giving rise to "puffing" may be noted. This phenomenon has been known since the time of Persoon (1798) and Desmazieres for the larger Discomycetes, and according to De Bary (6) simultaneous ejection of the spores from the

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ascus occurs in nearly all species of this class. The forcible expulsion of spores from perithecia was not observed however until a later time. Several of the noteworthy observations may be mentioned. Tulasne (20) observed the ejection of the spores from the perithecia of Claviceps in 1861; this was confirmed by De Bary (6) and the same phenomena noted for Cordyceps. The details of the process of spore expulsion were studied by Pringsheim (13) for *Sphaeria scirpi* in 1858; by Woronin (24) for *Sphaeria lemaneae* in 1869; by Wolff (23) for Erysiphe in 1875; by Zopf (25) for Sordaria species in 1880; while De Bary in 1884, in his discussion of the expulsion of spores, claims that the process is common in the Pyrenomycetes. It may be pointed out in this connection that ascospores are not infrequently set free from the perithecium by the mucilaginous swelling of the asci and are forced out embedded in mucilage in drop-like aggregations or in the forms of tendrils or spore-horns instead of being set free by the explosion of the asci. Pyrenomycetes showing the two types of spore expulsion have been designated as *active* and *inactive*. In the active forms, according to De Bary, the entire spore content of the ascus may be ejected *simultaneously* as first described by Zopf (25) for Sordaria, or the spores may be expelled in *succession* as was first shown by Pringsheim (13) for *Sphaeria scirpi*. In connection with this claim the more recent view of Falck (8) should be presented: "According to my investigations conducted on species from all classes, I can state it as a rule that in all active Ascomycetes the spores are shot out singly, that is, one spore after the other at regular intervals. The intervals are mostly so short that the ejection appears to the eye as single. In proper microscopic preparations in which the ejection is often retarded, one can follow directly how one spore after the other is emptied from the ascus."

According to all published observations, to be referred to later, the expulsion of the spores from the ascus in Endothia is *simultaneous*.

The forcible expulsion of the ascospores of the black rot fungus, *Guignardia bidwellii*, was noted by Scribner and Viala (18) and the following main facts established:

1. The ascus swells up to double length and is set free from the perithecium through the ostiole or by the rupture of the wall. 2. When the asci have escaped they expel the contained spores one at a time. 3. The maximum height of projection was 4 cm. 4. A temperature of 20°–30° C. was necessary for expulsion.

The importance of the ascus discharge in the dissemination of the black rot of the grape is brought out by Reddick (17) who has confirmed the observations of Scribner and Viala, and Wallace (21) has made a similar contribution in his study of the life-history of the apple scab fungus, *Venturia inaequalis.* Perhaps the most notable recent work is that of Falck (8) on the air infection of ergot, and the dissemination of infectious diseases of plants by means of convection currents ("Temperatur-strömungen"). In the course of this work Falck points out the effectiveness of convection currents in the dissemination of ascomycetous spores.

Expulsion of ascospores by the chestnut blight fungus.—The earlier reports on the chestnut blight fungus make no mention of the way in which the ascospores are set free from the perithecia. Although the first studies appeared in 1906 it was not until the summer of 1911 that Rankin (14) observed the forcible expulsion of the ascospores. Later (15) the same writer says: "Under moist conditions the ascospores are shot forcibly out in the air where they can be caught up by the wind and carried for a considerable distance. The speaker found the ascospores being shot from mature pustules during every rainy period last summer." Considering the fact that forcible expulsion of ascospores by many pyrenomycetous fungi was a fairly well-known phenomenon, as has been pointed out in the previous pages, it seems strange that this important observation did not come earlier. It was this which served as the impetus for investigations along new lines on the dissemination of the fungus.

Additional observations and experiments on the expulsion of the ascospores made at the Field Laboratory of the Pennsylvania Chestnut Tree Blight Commission during the summer of 1912 are summarized by Anderson (1) and presented in detail by Anderson and Babcock (2). The most important points brought out by these investigations were as follows:

1. Ascospore expulsion occurs only during and for a short period after rains or as long as the bark remains wet. 2. The maximum distance to which ascospores were expelled vertically was 22 mm., while the maximum horizontal distance was 89 mm. 3. The rate of ejection under favorable conditions was an ascus explosion about every two seconds or an average of 4.06 spores per second. 4. Bark bearing perithecia and kept moistened continued to expel ascospores for 25 days.

The way in which the ascospore expulsion occurs was pointed out by Rankin (16). According to his observations the asci are set free and rise in succession to the ostiole, where the explosion takes place, eight spores, or the entire contents of an ascus, being liberated at each pulsation. These observations were confirmed by Anderson (3) and in connection with the study of the morphology of the ascus, the mechanics of the spore discharge was briefly considered.

The senior author first pointed out the relation of temperature to the expulsion of ascospores (9), showing that moisture and the proper temperature are essential to the proper activity of the process. This relation was again emphasized (10) and the bulletin referred to contains the first published illustration of a characteristic spore print on an object slide. The first data on relation of temperature to ascospore expulsion were obtained from experiments conducted in constant temperature rooms, and these were substantiated later by tests carried out under field conditions (11). There was no expulsion of ascospores from Nov. 26, 1912, until the rain of March 21, 1913, although there were 21 warm winter rains during this period with maximum temperatures during or immediately following the rain of 35–60 degrees Fahr.

The length of time during which ascospore expulsion occurred following various warm rains was given special attention in some recent studies on air and wind dissemination of the chestnut blight fungus (12). It was found to depend upon the weather conditions following the cessation of a rain, and varied from 45 minutes to a maximum of 13 hours.

Our work on the relation of temperature to ascospore expulsion has been summarized by the junior author (22).

Two facts established by the researches of Falck have a very important bearing on the dissemination of the spores of Endothia. First, he has shown that "Temperatur-strömungen" alone suffice for spore dissemination of Claviceps; and for this reason fields protected by woods from strong winds and remaining moist longer than free lying fields are more infected. Second, he points out the effect of evaporation in the production of convection currents. It is certainly worthy of note that the ascospores of Endothia are being expelled under natural conditions in the field at a time when evaporation from the bark is favorable to the creation of convection currents (12). For this reason we chose to speak of the "air and wind dissemination" of the spores, since an evident wind is not necessary to scatter the spores, but only helps to secure a wider dissemination.

METHOD

A study of various phases of ascospore expulsion under artificial conditions has been carried out during the past year. A series of nine main experiments covering practically the entire year of 1913 was conducted. Specimens of excellent quality showing mature perithecial stromata were obtained at Emilie, Bucks County, Pa., and fresh material was always collected for each test. Great care was taken not to let the specimens dry out previous to starting the experiments.

In conducting the tests, small pieces of bark of approximately 3.5 × 1.5 inches were placed on two layers of blotting-paper in shallow granite trays, 8 × 12 inches. Six pieces of bark were usually used for each tray and in selecting the specimens care was taken to secure pieces with a uniform distribution of perithecial stromata. Control specimens were always used and whenever possible they were taken from the other half of pieces used for the test. If this was not possible pieces of bark of uniform character were selected. Slides were supported and fastened over the bark by the following method: Two pieces of match sticks of the same length as the width of the slides, were dipped in melted wax (beeswax and resin) and one placed across each end of a slide. The wax was allowed to harden and the sticks were held securely. When placed on the bark the slides were supported about 2–3 mm. above the perithecial necks and thus sufficiently near to catch the ascospores on their under surfaces whenever expulsion occurred. In order to support the slides over the same material each day, four to six pins were stuck into the bark at the sides and ends of the slides. Each time before moistening the specimens the slides were removed and water of the same temperature as the room or incubator chamber was sprayed on the surface of the specimens and the blotting paper was also thoroughly drenched. Records were taken once a day, that is, the individual spots, each of which represents an active ostiole, were counted and their density noted. Not less than twelve specimens were used for each experiment, except in one or two cases with minor tests, and a like number was used for the control. Complete temperature records were obtained for each experiment by the use of Friez thermographs.

THE RELATION OF TEMPERATURE TO ASCOSPORE EXPULSION

The relation of temperature to the forcible expulsion of ascospores from perithecia appears to have been passed over with only brief consideration. In a few investigations the relation of this process to proper temperature conditions has been mentioned. Scribner and Viala (18) stated that a temperature of 20–30 degrees C. was necessary for the forcible ejection of the spores of the black rot fungus, but gave no details of experiments upon which this assertion was based. Falck (8) in a discussion of the dissemination of Claviceps spores by convection currents, brings out the point that this phenomenon is least pronounced at cellar temperatures, and attributes this entirely to the lack of "Temperatur-strömungen," but unfortunately the activity of the process of expulsion was not taken into consdieration. It seems probable from our results that spore expulsion would be much less active at cellar temperatures. At another point he makes the statement that temperature has a pronounced effect on the rapidity of spore expulsion, increasing with rise in temperature, but no record is given of the temperatures at which observations were made.

No exhaustive attempt has been made to bring together the literature dealing with the relation of temperature to ascospore expulsion in general, but it can be definitely stated that no previous work bearing on the relation of temperature to ascospore expulsion in the chestnut blight fungus has been published.

In studying the relation of temperature to expulsion of ascospores of the blight fungus nine tests were carried out covering a range of temperature from 36.5° F. to 100° F. No expulsion was obtained at the lowest temperature employed but with each increase in temperature came an increase in expulsion until the optimum, 70°–80° F., was reached, beyond which expulsion gradually lessened. At 100° F. it had practically ceased. The nine tests were as follows:

(*a*) *Temperature 36.5°–40° F. Average 38.6° F.* The specimens were collected at Emilie, Bucks County, Pa., on Dec. 22, 1912, prepared by the method described, and the experiment started two days later with 21 pieces for the test and 23 for the control. The specimens were kept at this temperature in a cold room for 12 days where there was absolutely no expulsion. They were then moved to the laboratory where for the first three days they remained inactive, but after that time there was a gradual increase until at the end of two weeks all but one specimen had expelled ascospores. The expulsion was on the whole rather moderate. The control kept at laboratory temperature, about 72° F., shot abundantly.

(*b*) *Temperature 52.8°–54.9° F. Average 53.8° F.* The specimens were collected at Oxford, Chester Co., Pa., Dec. 30, 1912, prepared as described above and the experiment started Jan. 6, 1913 with 20 specimens for the experiment proper and 19 for the control. The trays were kept in a cold room for two weeks at an average temperature of 53.8° F. Shooting was very light, as may be noted from the following summary:

TABLE I

NUMBER OF SPECIMENS SHOWING EXPULSION AT 53.8° F.

No. of days shooting occurred	0	1	2	3	4
No. of specimens	6	5	4	2	3

It may be seen from this tabulation that 6 specimens showed no expulsion whatever for the entire period of two weeks, while the maximum amount was given by three specimens which gave expulsion on four different days. After the first two days only single spots were obtained from any specimens. At the end of two weeks they were moved to laboratory temperature, about 72° F. for 12 days where expulsion occurred from all but one specimen. Shooting was moderate. The trays were again moved to the cold room, this time for 13 days, at an average temperature of 54.9° F. For the first two days expulsion continued as at laboratory temperature but after this time it was very light and corresponded to the first two weeks in the cold room. The control at the laboratory temperature, average 72° F., shot abundantly.

(*c*) *Temperature 54°–59° F. Average 56.25° F.* The specimens were collected at Emilie, Bucks Co., Pa., Nov. 19, 1913, and prepared according to the regular methods, but the experiment was not started until Dec. 3, 1913. This delay was caused for the reason that the cold room had not been adjusted to the proper temperature. The trays for the experiment were put in the cold room and those for the control in the laboratory Nov. 20, 1913 and the blotting paper in the trays moistened each day to prevent the specimens from drying out. Dec. 3, 1913, the bark was wet and the test started with 12 specimens for the experiment and 12 for the control. The trays were kept in the cold room for 10 days at an average temperature of *56.25° F.*

Expulsion was light but all the specimens shot more or less. The control specimens at laboratory temperature, 70.8° F., gave an abundant spore expulsion.

(*d*) *Temperature 59°–65.5° F. Average 62.5° F.* The specimens were collected at Emilie, Bucks County, Pa., Feb. 6, 1913, and prepared by the usual method. From the time of collection until February 13 the specimens were kept moist. The bark was wet and the test started on this date but the trays were kept for two days at laboratory temperature to make certain that the material was in the proper stage of development for expulsion. On February 15, 1913, two trays were put in the cold room and two kept in the laboratory for a control. 12 specimens were used for the experiment proper, and 12 for the control. The test specimens were kept in the cold room at an average temperature of 62.5° F. for 17 days, during which time expulsion was light. All of the specimens showed some active ostioles. The control specimens shot abundantly. At the end of the 17 days, on March 4, 1913, the two cold room trays were put in a room held at a temperature of 24° F. for one week. The bark was of course frozen solid during this time. At the expiration of the week, they were again removed to the first cold room and observed for 19 days, or until March 31. The first day after removal from the 24° F. cold room into the 61.5° F. room all but four of the specimens showed expulsion, the highest number of spots for one slide being 52. On the second and third days there were no active ostioles and on the remaining 16 days very few in comparison with the first test of 17 days before being put in the room at 24° F.

(*e*) *Laboratory temperature, average 71.9° F.* All of the controls for the preceding and following experiments were run at laboratory temperature which averaged close to 72° F. At this temperature, as well as anywhere between 68° and 80° F., expulsion seems to occur very abundantly, whenever the proper moisture conditions are supplied. There does not appear to be any definite temperature which might be called the optimum or even any sharply defined limits. A few degrees one way or the other do not seem to make any appreciable difference. However, between the above stated temperatures, expulsion appears to take place most freely (see Table II).

(*f*) *Temperature 76.5°–80.5° F. Average 79° F.* The specimens were collected at Emilie, Pa., Nov. 5, 1913. The same methods were used as described above except that a Prairie State chicken incubator was used to furnish the required temperature. 12 specimens were used for the experiment proper and 12 for the control. Two trays of specimens were put in the incubator on Nov. 6, 1913, 3 hours previous to wetting the bark and starting the experiment. This was done to equalize the temperature of all. A thermograph and a beaker of water for use in moistening the specimens were also kept in the incubator. The test was started at 3:00 P.M., on November 6 and continued for 9 days at an average temperature of 79° F. At this temperature the specimens seemed to shoot abundantly and just as actively as the control specimens. There was but little appreciable difference either in density or number of the spots; although the number of active ostioles per trap per day for all controls was 72.6, and 84.5 for the specimens at 79° F. (see Table II).

(*g*) *Temperature 82°–86.5° F. Average 84.6° F.* Specimens were collected at Emilie, Pa., Nov. 19, 1913, the customary methods used in their preparation, and the experiment started November 20. The same incubator was used as for the preceding (79°) experiment. The trays of specimens were put in the incubator at 3:30 P. M. in order to equalize the temperature but the bark was not drenched with water or slides adjusted until 10:00 A. M. The test was continued for 11 days, during which time all of the 12 specimens showed active ostioles but expulsion was rather moderate and could not be termed abundant except in some few individual cases on certain days. The control specimens shot abundantly. For the first eight days, while the amount of expulsion from the control specimens exceeded that from test specimens, it was more noticeable during the last three days when the effect of the high temperature began to be very evident. After the first day the density of the spots was always greatly in favor of the control. On the whole the amount of expulsion at 84.6° F. should be characterized as medium in comparison with the abundant expulsion at the more favorable temperature (see Table II).

(*h*) *Temperature 89°–92.5° F. Average 90.8° F.* Specimens were collected Oct. 23, 1913, at Emilie, Pa., and prepared the same as the preceding ones. 12 specimens were used for the experiment proper and 12 for the control. An incubator was used as in the two preceding tests, to obtain the desired temperature. The specimens were prepared on October 24 and kept moist until October 27 by wetting only the blotting paper. Absorption from the paper however furnished sufficient moisture in 17 out of the 24 specimens to cause considerable spore expulsion. Two trays were put in the incubator at 11:00 A. M., on October 27, in order to equalize the temperature but the specimens were not wet until 5:00 P. M. the same day. The test was continued for 7 days in the incubator. On the first day at this temperature 8 traps showed expulsion averaging about 70 spots per trap. However, after the first day only three spots were noted and these from 2 slides on the last day. The abundant shooting on the first day in the incubator was probably due to the fact that the specimens had been shooting rather abundantly for two days previous to starting the experiment at this temperature. In comparison with the control which shot abundantly, there was a very light expulsion at 90.8° F. (see Table II).

(*i*) *Temperature 97°–100° F. Average 98.4° F.* The specimens were collected at Emilie, Bucks County, Pa., prepared by the same method as for the preceding tests, and the bark wet and experiment started April 2, 1913. 12 pieces of bark were used for the experiment proper, and 12 for the control. All of the specimens were kept at laboratory temperature for the first day and on April 3 half were put in the high temperature room and the others retained at the laboratory as controls. The test was continued for 15 days at an average temperature of 98.4° F. For the first 5 days the specimens were wet once a day but this was not sufficient because of drying out due to the high temperature. Only two active ostioles were recorded during this 5-day period and those from a single specimen on the fourth day. After the fifth day and until the 15th, when the test was brought to a close, the specimens were moistened twice a day and not allowed to dry out. On the sixth day 8 of the traps shot spores but expulsion was very light, there being only 41 spots from all. On the 7th and 8th days three specimens showed expulsion of spores but there were less than 20 spots each per day. For the remaining 7 days, there was absolutely no expulsion. The traps were then moved to the laboratory and kept under observation for 22 days. For the first 14 days there was practically no expulsion but after that they gradually picked up and on the last 3 days of the test every specimen shot rather freely. The control traps at laboratory temperature averaging 70.3° F. showed abundant expulsion, or an average of 64.6 active ostioles per specimen per day, while the specimens at 98.4° gave an average of only 0.4 (see Table II).

The results of the preceding tests on the relation of temperature to ascospore expulsion are summarized in Tables II and III.

Under artificial conditions in the laboratory expulsion of ascospores is entirely inhibited at low temperatures. With rise in temperature expulsion begins and activity of this process increases until finally expulsion of spores becomes abundant at temperatures ranging from about 68° to 80° F. At higher temperatures there is a gradual cessation of activity until at body temperature it is reduced to practically nil.

TABLE II

SUMMARY OF TESTS ON EFFECT OF TEMPERATURE ON ASCOSPORE EXPULSION

No. of Traps Used	No. of Days Tested	Temperature F.	Total No. of Spots Recorded	Av. No. of Active Ostioles per Trap per Day
21	13	38.6	0	0
23	13	72.5	14,424	48.2
20	14	53.8	408	1.4
19	14	72	6,627	24.9
12	10	56.25	1,503	12.5
12	10	70.8	12,969	108
12	17	62.5	3,609	17.7
12	17	72.8	16,871	82.7
12	9	79	9,125	84.5
12	9	72.2	8,950	82.8
12	11	84.6	4,232	32.06
12	11	72.2	9,664	73.2
12	7	90.8	462	5.5
12	7	72.5	8,112	96.5
12	15	98.4	74	0.4
12	15	70.3	11,643	64.6

It is interesting to note that there is a somewhat parallel relation between temperatures favorable for growth of the blight fungus and temperatures favorable for ascospore expulsion. The minimum, optimum and maximum temperatures for growth as given by Shear and Stevens (19) are as follows:

	Centigrade	Fahrenheit
Minimum..................	8–9	46–48
Optimum..................	18–28	64–82
Maximum..................	35	95

TABLE III

AMOUNT OF ASCOSPORE EXPULSION AT DIFFERENT TEMPERATURES

Specimen and Trap Numbers	Average Temperatures F.	No. of Active Ostioles per Trap per Day	Amount of Expulsion
11–18; 26–38	38.6	0	None
52–71	53.8	1.4	Very light
293–304	56.25	12.5	Light
150–161	62.5	17.7	Light
	71.9	72.6 Average for all controls	Abundant
245–256	79	84.5	Abundant
269–280	84.6	32.06	Medium
233–244	90.8	5.5	Very light
	98.4	0.4	Traces

It would appear from this comparison that continued ascospore expulsion is dependent to a certain extent upon growth. This is also substantiated by the tests made on the duration of ascospore expulsion as recorded in the following pages.

DURATION OF ASCOSPORE EXPULSION

Probably one of the most remarkable facts to be noted in regard to ascospore expulsion under artificial conditions is the power which the perithecial stromata have to expel spores for an almost indefinite period, provided they are given the proper temperature and moisture conditions.

On December 24, 1912, 23 specimens collected at Emilie, Pa., two days before, were prepared and a duration experiment started at laboratory temperature using the same method as for the temperature tests. Daily records were taken for 5 months and 17 days and at the end of this time, June 9, 1913, all but three of the specimens showed some active ostioles. One of these three specimens was overgrown by molds and undoubtedly had ceased shooting permanently but the other two, according to their former records, would probably have started up again. One peculiarity of ascospore expulsion is the fact that when first brought in from the field, the perithecia will generally shoot spasmodically for some days, that is, they will expel spores abundantly for one, or sometimes two or three days in succession, and then drop off to a very small amount. Then the succeeding days they will either gradually pick up and shoot abundantly for two or three days or expel at their maximum capacity the first day after the rest period. The length of this spasmodic expulsion varies with the specimens used. Sometimes it continues for two or three weeks and sometimes for only a few days. At the end of this type of expulsion comes a rest period when the specimens may either cease entirely or reduce expulsion to a minimum. This may continue for a short or long time. In one case shooting ceased entirely for 7 weeks after the spasmodic period, and then gradually picked up and for over 7 weeks shot abundantly without missing a day. Table IV shows for each specimen the number of days expulsion occurred during the entire period, the number of days when there was no expulsion, the total number of spots recorded and the average number of active ostioles per day for the days when expulsion occurred.

The records summarized in this table certainly indicate an almost phenomenal power of ascospore production by the chestnut blight fungus. The short period of continuous ascospore expulsion recorded by Anderson and Babcock (2) was but a meager expression of a most persistent power. These tests under laboratory conditions seem to point to the probability that perithecial stromata under natural conditions in the field will not exhaust their power to produce and eject ascospores within the limits of a single season. Field tests in progress will shed definite light on this point. We may point to this remarkable power as one of the several factors that combine to make the chestnut blight fungus the most pernicious parasite that has ever invaded our forests.

Effect of Removal of Perithecial Necks

Three experiments were carried out at laboratory temperature to determine what effect the removal of the perithecial necks would have on the expulsion of ascospores. The tests were as follows:

Two specimens collected at Emilie on Dec. 22, 1912, were used, one for the experiment and one for the control. Both had the perithecial necks well developed; in fact one large piece of bark was broken into two parts so that the material would be in the same stage of development. On one specimen, every neck, with part of the stromatic tissue beneath, was removed with a sharp razor. The specimen was examined thoroughly with a strong lens to make certain that there were no necks remaining. On Dec. 24, 1912, at 10:00 A. M. both pieces of bark were wet and slides adjusted over them. On the 3d day the specimen with the necks removed shot 1 spot whereas the control in the meantime had expelled 77 spots. Examination showed that the necks were reforming and from the third day on expulsion from the regenerated necks was as good as from the control.

TABLE IV

SUMMARY OF TESTS ON THE DURATION (168 DAYS) OF ASCOSPORE EXPULSION

No. of Trap	No. of Days on which Expulsion Occurred	No. of Days on which there was No Expulsion	Total No of Spots Recorded	Average Number of Active Ostioles per Day for Days when Expulsion Occurred
4	128	40	721	5.6
5	164	4	12,140	74.0
6	145	23	4,893	33.7
7	143	25	4,266	29.8
8	73	95[1]	1,721	23.6
9	148	20	5,742	38.8
10	153	15	11,203	73.2
19	154	14	9,834	63.9
20	126	42	3,196	25.4
21	147	21	9,574	65.1
22	166	2	24,018	144.7
23	168	0	18,079	107.6
24	139	29	1,906	13.7
25	154	14	10,004	65.0
39	154	14	5,979	38.8
40	165	3	16,363	99.2
41	153	15	3,848	25.2
42	152	16	20,323	133.7
43	156	12	9,533	61.1
44	149	19	7,344	49.3
45	154	14	9,295	60.4
46	146	22	9,313	63.8
47	108	60	10,232	94.7

On Dec. 28, 1912, the necks were removed on two more specimens. In one case the specimen with the necks removed shot after 4 days. In the other case the specimen with the necks removed did not shoot for 7 days when 50 spots were recorded while the check shot the 4th and 5th days with 10 and 18 spots respectively.

On Feb. 12, 1913, the necks on 4 more specimens were removed and the test started with a check for each. In three cases the control specimens showed expulsion before those from which the necks had been removed. The latter averaged 4 days before any expulsion occurred. In the 4th case both control and experiment proper shot on the 3d day.

While the number of tests is hardly sufficient to warrant a final statement it seems highly probable that the structure of the perithecial necks has much to do with the conduction of the asci to the ostiole where the expulsion takes place. It has been pointed out by Anderson (3) in his study of the structure of the perithecium that the periphyses of the neck "act as so many little springs" and press the asci back. It may be added that the ascus is also held in position and any back movement prevented by the periphyses which spring back after its passage through the neck. This function of the periphyses would explain the results obtained for spore expulsion when the perithecial necks are removed. The time required for the necks to regenerate varied from 3 to 5 days.

Ascospore Expulsion from Inverted Perithecia

In considering ascospore expulsion one of the questions which very naturally presented itself was how the asci arrived at the end of the long neck. It was conceivable that detached asci might float to the top of the neck in the water with which it appears to be filled when expulsion is taking place. As a result of a study of the morphology of the perithecium Anderson (3) claims that the asci are forced to the ostiole as a result of expansion due to water absorption, and then that the supply is kept up by the growth and maturing of new crops.

In order to determine whether ascospore expulsion would take place when the necks were pointing downward, two tests were made in which the bark bearing the stromata was placed in an inverted position. These two tests were as follows:

On Jan. 11, 1913, a two-inch branch showing well-matured perithecial material, collected at Emilie, Pa., was soaked in tap water for 20 minutes with the necks inverted. At the end of this time an object slide was adjusted to the under surface of the branch, so as to be 2–3 mm. below the ostioles. The bark on the upper part of the branch was peeled off so that water could be injected between the wood and bark and be absorbed by the inverted perithecia below. The bark was kept moist and the test run for 8 days. Expulsion was light but was obtained on every day except one, the highest number of spots being 25.

On Feb. 13, 1913, 6 flat pieces of bark from larger branches were treated similarly to the above tests except that wet blotting paper was kept on top of the specimens to insure sufficient moisture for expulsion. The test was continued for 11 days and shooting was recorded from all but one specimen. Expulsion was moderate except on two days from the same piece when the spots were very abundant. One peculiarity of the spore prints from inverted specimens is that the majority of the spots are not round and clearly defined as they usually are when the spores are shot upward (fig. 1). The spot is generally elongated, denser at one end, and fades out at the other somewhat like a comet's tail (fig. 2). The explanation for these is probably the fact that many of the long perithecial necks are not in a vertical position but grow obliquely. When expulsion starts it is natural to suppose that the spores are shot out more forcibly and at that time a more or less distinct spot is formed. However, as shooting continues, the spores are ejected with less force and they fall short of the original spot and form the "comet's tail." When the necks point upward,

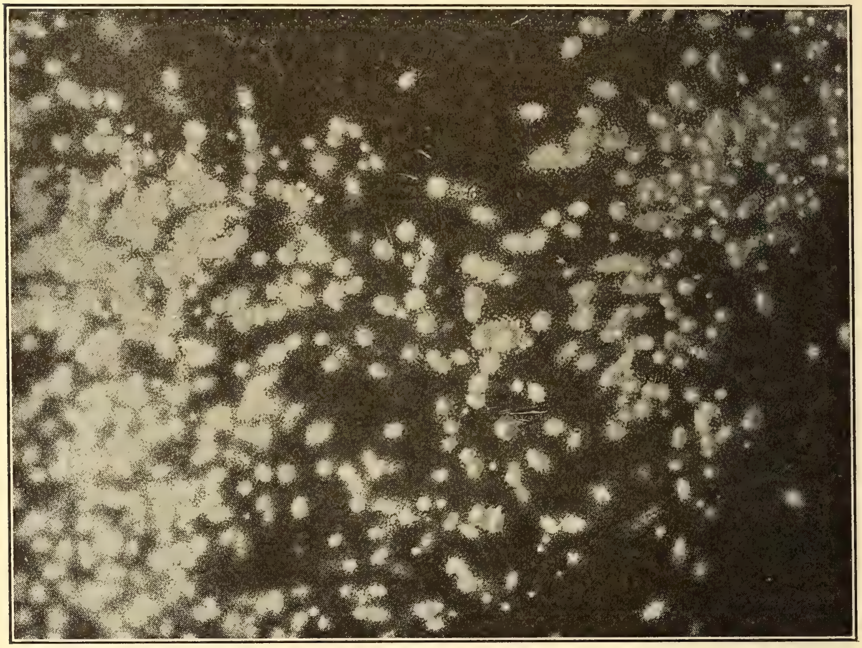

FIG. 1. A characteristic spore print of *Endothia parasitica* on an object slide, obtained when expulsion of the ascospores was taking place under optimum conditions.

only a few "comet's tails" are formed and this is probably when the slide is of medium distance from the ostiole. Unless the slide is very close spores from obliquely directed necks never reach the slide.

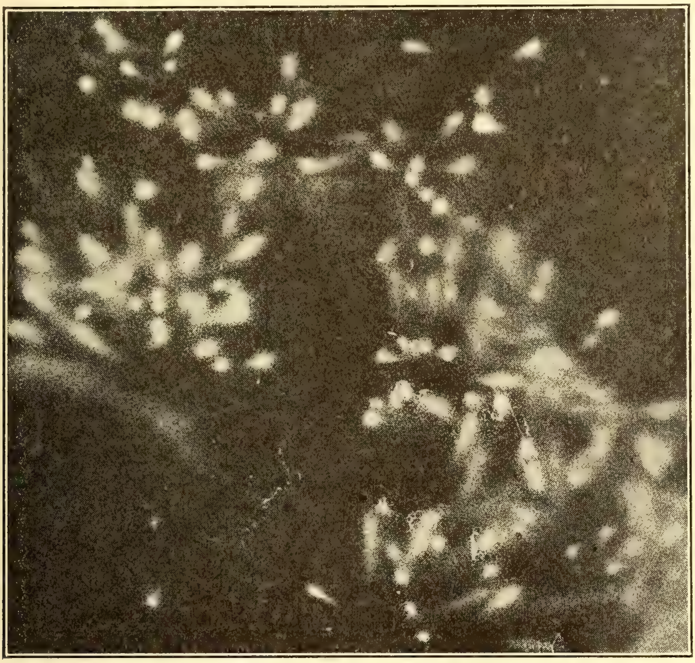

FIG. 2. Spore print of ascospores of *Endothia parasitica* obtained from perithecia in an inverted position. The comet-like spots are very characteristic.

THE EFFECT OF SATURATED ATMOSPHERE ON ASCOSPORE EXPULSION

In order to determine the effect of a saturated atmosphere upon ascospore expulsion, two tests were run at laboratory temperature. The specimens used were collected at Emilie, Pa. The experiments are as follows:

Three specimens of excellent perithecial material were selected for the test and two of them put in a large closed damp chamber on wet blotting paper while the third was kept as a check in an open chamber. The specimens were wet, slides adjusted and the experiment started Jan. 11, 1913. Records were taken for 27 days at the end of which time the control was found to have shot 71 spots to an average of 133 for the other two.

A more significant test because of the greater number of specimens was started Feb. 13, 1913, in damp chambers similar to those above. It was continued for 17 days with 6 specimens for the experiment proper and 6 for the control. At the end of the test it was found that while the checks showed a larger number of spots than the experiment specimens there was sufficient shooting from the latter to prove conclusively that expulsion will take place rather freely when the perithecia are in a saturated atmosphere.

This would point to ascospore expulsion as the result of high osmotic pressure from water absorption rather than to a drying effect from contact with the air as has been suggested by some writers (6). It should be pointed out in this connection that ascospore expulsion takes place during a rain under natural conditions, but continues for only a short period after the cessation of rain, the time varying with the humidity of the atmosphere.

Expulsion Without Direct Wetting

Two tests were conducted to determine whether ascospore expulsion could take place without direct application of water to the upper surface of the bark. They were as follows:

Specimens obtained from West Grove, Pa., were arranged on blotting paper in trays in the customary manner. Six were used for the experiment proper and six for the control. Water was applied every day to the blotting paper beneath the test specimens and care was taken not to give an excessive amount or to wet the upper surface of the bark, while the control specimens were drenched from above in the regular manner. The test was continued for 17 days at the end of which time all but one of the experiment specimens had expelled spores. Expulsion however was very light but the controls also showed but few active perithecia. While positive results were obtained they were not as conclusive as might be desired because the specimens were of poor quality. Practically all of them became contaminated with bacteria, and the perithecia appeared to be rather immature.

On Feb. 12, 1913, another test similar to the above was started using specimens from Emilie, Pa. Six traps were used for the experiment and six for the control. Daily records were taken for 16 days during which time there was some heavy ejection of spores from the specimens which did not receive direct application of water. The control specimens however showed a still heavier expulsion. In all cases the water was applied to the blotting paper beneath the specimens and hence the only possible way by which it could reach the ostiole and cause expulsion was by absorption from below. This experiment was carried out mainly to determine whether ascospore expulsion would be likely to take place from stromata on logs or fallen limbs that might be left lying for some time in contact with the moist ground.

EXPULSION FROM ISOLATED PUSTULES

A determination of the amount of expulsion from isolated pustules was attempted but the results obtained were not entirely satisfactory due principally to contaminated specimens. Two tests were conducted as follows:

Material obtained Jan. 18, 1913, from West Grove, Pa., was used and 16 pustules were isolated by cutting away all perithecial necks in the immediate vicinity. Then in order to make certain that no shooting would occur from regenerated necks, a mask was made of paper by cutting a hole, the same size as the pustule and slipping it over the latter. Nine of the 16 pustules became contaminated and gave no results. The remaining ones were kept under observation for 43 days. The number of necks to each pustule was counted and the maximum number of spots recorded on any one day is indicated in Table V.

TABLE V

EXPULSION FROM ISOLATED PUSTULES

No. of necks per pustule	32	37	60	38	13	27
Maximum no. of spots obtained in one day	17	19	12	27	12	9
Percentage of active ostioles	53.1	59.3	20	71	92.3	33.3

A set of specimens similar to the above was started Feb. 15, 1913, and run at laboratory temperature for 41 days, except 4 pustules which were discontinued at the end of 22 days. The material was obtained at Emilie, Pa., on February 6. The same method was used as in the preceding test and 23 pustules were isolated. Table VI shows the results of this experiment.

It may be noted that pustules with necks ranging in number from 1–60 were used, and that activity from all ostioles was rare during any one day. The length of time a single perithecium will continue to expel spores has not yet been determined.

EFFECT OF ALTERNATE WETTING AND DRYING UPON ASCOSPORE EXPULSION

A test was started at laboratory temperature April 2, 1913, for the purpose of determining the effect of desiccation upon ascospore expulsion. Material from Emilie, Pa., was used and consisted of 5 sets of specimens with 3 to a set. No. 1 was wet and examined every day, No. 2 was wet every 2d day, No. 3 every 3d day, No. 4 every 5th day and No. 5 every 7th day.

The test was conducted for 42 days. The set which was allowed to dry one day gave by far the best expulsion. Shooting continued heavy to the end of the test and on the whole was considerably better than from the set which was wet every day. The sets wet every 3d, 5th, and 7th day respectively gave poor expulsion. One day of drying gives optimum results but thereafter the longer the period of desiccation the fewer the number of spots. The effect of alternate wetting and drying is shown by Table VII.

TABLE VI

EXPULSION FROM ISOLATED PUSTULES

No. of Necks per Pustule	7	5	7	5	5	7	7	60	1	37	5	47	24	47	15	19	19	19	29	34	19	23	18
Maximum No. of Spots Obtained in One Day	2	5	4	0	0	5	5	29	0	1	4	40	18	34	14	15	1	10	15	13	15	15	11
Percentage of Active Ostioles	28	100	57	0	0	71	71	48	0	3	80	85	75	72	93	79	5	53	52	38	79	65	61

TABLE VII

EFFECT OF ALTERNATE WETTING AND DRYING ON SPORE EXPULSION

Time Moistened	Amount of Spore Expulsion
Every day	Abundant first week; moderate thereafter
Every 2d day	Heavy expulsion every day
Every 3d day	Light
Every 5th day	Very light
Every 7th day	Practically none

Field tests already carried out show that these results are no indicator of what may be expected under natural field conditions.

SUMMARY

1. Under artificial conditions in the laboratory spore expulsion is entirely inhibited at low temperatures. At higher temperatures there is a gradual increase in expulsion, with the optimum between 68° and 80° F. Spore expulsion was tested at temperatures ranging from 36° to 100° F. The results obtained in the laboratory substantiate the field results which have shown the cessation of spore expulsion during the winter period.

2. Perithecia show an almost phenomenal power of spore production, as shown by the fact that spores were expelled from some specimens every day for 168 days. Some perithecia were still active when the test was discontinued.

3. The necks of the perithecia play an important part in the mechanics of spore expulsion. Perithecia from which the necks have been removed cease to expel spores. Under favorable conditions for growth the necks will be regenerated, and with the formation of new necks spore expulsion is resumed.

4. Expulsion continues from inverted perithecia, the spore prints having a characteristic form. The asci are apparently brought to the ostiole as a result of pressure within the perithecium.

5. Spore expulsion will occur in a saturated atmosphere but appears to be more pronounced when specimens are permitted to dry out gradually.

6. Direct wetting of the stromata is not necessary for spore expulsion, but sufficient moisture may be absorbed from below. This points to the probability that fallen logs or bark bearing perithecial pustules may absorb sufficient moisture from the wet ground to cause spore expulsion.

7. In general all of the perithecia of a pustule are not expelling spores at the same time. The reverse is sometimes true for small pustules, but for larger pustules the maximum number of active ostioles varies from 30 to 90 per cent of the total.

8. Even under laboratory conditions, alternate wetting and drying does not inhibit the process of spore expulsion, but the best results are obtained from specimens moistened every other day.

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GLIDING GROWTH AND THE BARS OF SANIO

J. G. Grossenbacher

Introduction

By gliding growth is meant the independent elongation or enlargement of cells in the growing zone which occurs, as some maintain, during differentiation and radial growth. The bars of Sanio are said to consist of cellulose and pectic materials in the form of bars or imperfect tubes which extend through two or more cells arising from the same initial in a direction at right angles to the plane of division. These bars have been studied chiefly as they occur in the xylem and phloem of trees, but have also been shown to occur in the more superficial cells of woody plants, where they extend parallel to the surface instead of as radii, as is the case with those arising in the cambial zone of trees.

The bars arising in the cambium have been brought into discussions to refute the contention that certain cells on arising from the cambium undergo elongation during differentiation, as well as in studies on the phylogenetic relationship of some Coniferae. In investigations into the nature and activity of the cambial sheath the bars have also been used. It seems, therefore, that gliding growth and the bars of Sanio have been of much interest to botanists during many years. The present discussion aims to contribute some concrete evidence on the subject of gliding growth and at the same time to make brief mention of the related literature.

Some Historical Aspects

Most of the earlier investigators who incidentally studied this matter in connection with the differentiation of the cells from the cambium infer that gliding growth occurs, but their inferences were usually not based on sufficient tangible data.[1]

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[1] Trécul, A. Origine et development des fibres ligneuses. Ann. Sc. Nat. Bot., 3. Ser. **19**: 63–74. 1853. Hartig, Th. Ueber die Entwicklung des Jahrringes der Holzpflanzen. Bot. Zeit. **11**: 553–60; 69–79. 1853. Velten, W. Ueber die Ent-

Krabbe[2] made a special study of the subject of gliding growth and found that it is typical of the cells differentiating to form the characteristic cellular elements of fibrovascular bundles in both monocotyledons and dicotyledons. He found that in the case of certain monocots practically the entire xylem of the secondary fibrovascular bundles arises from single vertical rows of cells which grow many times their original length gliding past each other, thus producing a bundle instead of a row of cells. He holds, however, that cell elongation occurs only while the cells adjoining are still growing. The same was maintained by him regarding the special enlargement of certain cells to form vessels; *i. e.*, gliding growth in the transverse direction. Mischke,[3] as well as Nathansohn[4] later published some inferential corroborative evidence supporting Krabbe's findings. A more recent substantial contribution to the subject of gliding growth is by Jost.[5] In his study of the behavior of the cambium in crotches and where branches arise from a trunk, he has concluded that although continued increase in thickness of the two components at such branching points necessitates a reduction in area of the cambial mantle or sheath, no cambium is actually eliminated. He therefore assumed that the cells of the cambium glide between each other as the area to be covered becomes smaller and that the cytoplasmic connections obtaining between the cambial cells are re-established at the conclusion of the gliding growth. He also thinks that primary rays of stems are divided and the parts displaced laterally to form many narrow rays by the gliding of the cambial cells in such a way as to break up the very broad primary rays.

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[2] Krabbe, G. Das gleitende Wachsthum bei der Gewebebildung der Gefässpflanzen, pp. vii + 100, pl. 7, 1886, Berlin.

[3] Mischke, K. Beobachtungen über das Dickenwachstum der Coniferen. Bot. Centralbl. **44**: 39–43; 65–71; 97–102; 137–42; 169–75. 1890.

[4] Nathansohn, A. Beiträge zur Kenntniss des Wachsthums der trachealen Elemente. Jahrb. Wiss. Bot. **32**: 671–86. 1898.

[5] Jost, L. Ueber einige Eigenthümlichkeiten des Cambiums der Bäume. Bot. Zeit. **59**: 1–24. 1901.

A paper by Klinken[6] has just appeared, in which the subject of gliding growth is discussed from various angles. The study was based on serial and other radial sections of Taxus taken through the cambial region, and including both xylem and phloem. Longitudinal gliding growth was found to occur in the xylem but not in the phloem. The cambium cells were found to undergo unlimited gliding growth or independent elongation. They were found to undergo transverse divisions after attaining certain lengths. Some of his published figures also indicate that some of the xylem cells coming into contact with medullary rays are often bent and curve somewhat to one side.

Neeff[7] recently contributed some interesting observations on gliding growth. He noticed that in the wood the ends of some cells were often compressed between others where resistance to elongation appears to have been too great. A figure is given showing a case where two cells are being forced apart by an intruder; the two halves of a pit are forced apart. See figure 6, which is copied from Neeff. Similar occurrences were also found in the phloem, as shown in the same figure noted above. He also found cases in which pits, corresponding to those that had been displaced by gliding growth, had apparently been formed in the walls of new neighbors. On the other hand, instances were also noted in which the pit connections existing between cells prevented gliding growth or retarded it to such an extent as to cause a decided compression or puckering of the growing cells.

The fact that the bars of Sanio occurring in the most closely related cells of a radial row always form a continuous and unbroken bar is regarded as indicative that gliding growth is of very minor importance or is wholly lacking. According to Raatz[8] the running of bars through only one or a few cells is indicative that the meristematic cell in which such bars originated underwent only a limited number of divisions before being forced out of the cambial zone by the progressive differentiation of the cells in the cambium sheath. The long bars that extend through one or more season's growth, on the other hand, are said to have arisen in a meristematic cell in the most active part of the cambial sheath and which therefore continued to give rise to new xylem and phloem cells during a considerable period before its last derivative arrived at a position in the cambium where it was forced to become differentiated into a mature cell and cease further division. Such an argument not only tends to show that cells through which these bars extend have not glided on each other, since they arose from each other by division, but also indicates, as Raatz and later Nordhausen,[9] Schoute,[10] and Müller[11] maintained, that Sanio's simple hypothesis on the nature and method of origin of cells from the cambium does not cover the facts of cambial division as it occurs in our trees. Incidentally it may also be mentioned that since Raatz and others found these bars occurring in numerous and widely separated species of trees, and Petri[12] has more recently shown them to occur in the cultivated varieties of the grape when affected by a disease known as "courte noue," they seem to have very little of phylogenetic significance in the conifers as maintained by Gerry[13] and others. Groom and Rushton[14] have recently also contributed to the question of the bars or rims of Sanio.

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[6] Klinken, J. Über das gleitende Wachstum der Initialen im Kambium der Koniferen und den Markstrahlverlauf in ihrer sekundären Rinde. Bibliotheca Botanica 19: Heft 84, pp. 41, 1914.

[7] Neeff, F. Über Zellumlagerung. Ein Beitrag zur experimentellen Anatomie. Zeitschr. Bot. **6**: 465–547. 1914.

[8] Raatz, Wilh. Die Stäbbildungen im secundären Holzkörper der Bäume und die Initialentheorie. Jahrb. Wiss. Bot. **23**: 567–636. 1892.

GLIDING GROWTH IN APPLE (*Pyrus malus* L.)

While studying the development of the initial stages of crown-rot of apple trees in cross sections it was frequently noticed that the more or less regular radial rows of wood cells arising from corresponding rows in the cambial zone are separated by cells in their angles. The diameter of the intruding cells nearest the cambium was usually very slight and increased as the distance from the cambial zone increased. These intercalated cells were suspected of being some that had entered the level of the section from one side or the other by growing in length and penetrating between the ends of the neighboring cells. Such an idea seemed plausible because of the fact that the intruded cells nearest the cambium are small and no corresponding radial rows could be found in the cambial zone from which they could have arisen. The absence of a corresponding row from the phloem also showed that such cells present in the xylem could not have arisen from an eliminated cambial row.

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[9] Nordhausen, M. Zur Kenntniss der Wachstumsvorgänge im Verdickungsringe der Dikotylen. Beiträge Wiss. Bot. 2: 356–400. 1898.

[10] Schoute, J. C. Über Zellteilungsvorgänge im Cambium. Verhandel. K. Akad. Wetensch, Amsterdam 9: Sect. 2, pp. 66, 1902.

[11] Müller, C. Ueber die Balken in den Holzelementen der Coniferen. Ber. Deut. Bot. Gesellsch. 8: (17)–(46), 1890.

[12] Petri, L. Ricerche sulle cause dei deperimenti delle viti in Sicilia. I. Contributo allo studio dell' azione degli abbassamenti di temperatura sulle viti in rapporto all' arricciamento. Mem. R. Staz. Pat. Veg., 1912, pp. 212, Roma.

[13] Gerry, E. The Distribution of the "Bars of Sanio" in the Coniferales. Ann. Bot. 24: 119–23. 1910.

[14] Groom, P., and Rushton, W. Structure of the Wood of East Indian Species of Pinus. Jour. Linn. Soc. Bot. 41: 457–90. 1913.

In case wood cells undergo longitudinal growth after arising from the cambium, it appeared likely that in occasional sections one ought to find unmated pits in cells that had been forced apart, for, as has been well established, many of the pits are derived from the cambium cells by division and must therefore be present on cell walls before gliding growth has been completed. As a matter of fact, several such instances were found, one of which is reproduced in figure 1. The fourth cell from the left in the lower radial row (2) of this figure shows an unmated pit, although the corresponding cell of the next row (1), that evidently arose from a cambium cell at this level, shows no corresponding pit. Along the upper edge of the figure is a medullary ray and on the left are the last wood cells of two radial rows that had been differentiated from the cambium. Between the two radial rows, arising from the cambium at the left, are seen what appear to be intruding cells (i) mentioned above. These intruders seem to have forced their way between their neighbors at the angles, at points where one usually finds angular intercellular spaces.

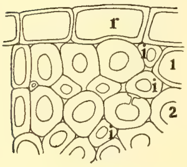

FIG. 1. *Cross Section of Apple Wood,* showing cells nearest cambium at left and a monoseriate medullary ray along the upper edge of figure. *r*, medullary ray. *1* and *2*, regular rows of xylem cells derived from the cambium at this level. *i*, cells intruding between the regular rows at their angles.

Longitudinal radial sections, however, furnished the most direct and therefore the most conclusive proof yet published to show that gliding growth occurs. A careful examination of many slides showed that the ends of wood fibers are often compressed and diverted at medullary rays, as had been found by Neeff. In some cases not only the ends of the wood cells in contact with medullary rays were flattened and enlarged, but the ray cells encountered were also compressed. In other instances the ends of the wood-cells were found diverted to one side into a direction more or less parallel to that of the rays, as may be seen in figure 2. Much variation was evident in the length of that portion of cells that was diverted from the direction of the other wood cells toward that of the rays; in one instance the diverted portion was found to measure 15.7 μ in length (figure 3). In several instances pits were found on the convex side of such bent wood cells in positions where the cell was not in contact with any other. Such cases were found in the bent groups outlined in figures 2 and 5.

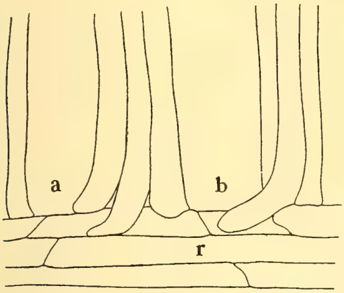

FIG. 2. *Longitudinal, Radial Section of Apple Wood.* *a* and *b*, large vessels. *r*, medullary ray.

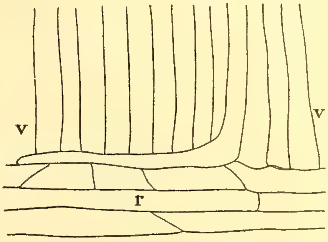

FIG. 3. *Longitudinal Section of Apple Wood,* showing one cell that has undergone considerable independent elongation. *v*, large vessels. *r*, medullary ray.

The diverted tips seem to have grown in various directions. In figures 3 and 5 the growth was along the ray toward the bark, which

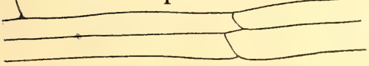

FIG. 4. *Section of Apple Wood,* showing various degrees of elongation of xylem cells. *v*, large vessel. *r*. medullary ray.

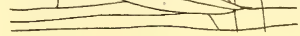

FIG. 5. *Section of Apple Wood,* showing a group of xylem cells diverted at end by encountering a medullary ray, *r*.

appeared to indicate that the gliding was toward the less lignified cells; but in the cases shown in figures 2 and 4 the matter is different. In the instance figured in 2, the ends of the cells curved toward the large vessels (*a*) and (*b*), while in 4 the curvature was away from the bark. It is seen to be in the direction of a vessel (*v*) that is rather remote from the starting point of the long glide.

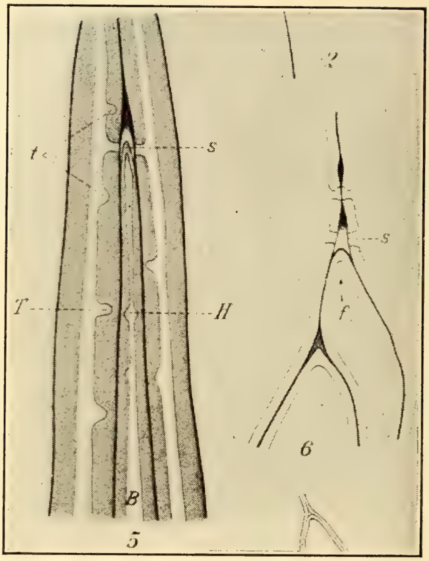

FIG. 6. *Longitudinal Sections, showing Gliding Growth.* *B*, elongating phloem cell intruding between two neighbors and forcing them apart, as shown at the pits. *f*, elongating xylem cell intruding between and forcing apart two other cells, as shown at the pit *s*. (Figures copied from Neeff's article, Über Zellumlagerung Zeit. für Bot. **6**: 465–547. 1914.)

GENERAL DISCUSSION

It appears to be an established fact that the Bars of Sanio run continuously through certain radial groups of cells in the wood and bark; and that such groups probably arose from the divisions undergone by a meristematic cell and its progeny subsequent to the origin of the bar, and before the last daughter cell of the group is forced from the cambial zone and becomes differentiated into mature tissue. The fact that the bars present in the different cells of such a group form an unbroken bar at maturity is not conclusive proof that the cells of such groups could necessarily not have undergone gliding growth; for it seems likely that the differential gliding growth takes place chiefly at the ends of the cells. The evidence recently given by Klinken, Neeff, and that found in apple appear to add sufficient direct proof to that formerly published to show beyond a doubt that gliding growth occurs among the differentiating wood cells of trees. The fact that pits in wood are often found to open into intercellular spaces instead of meeting corresponding pits in neighboring cells also shows that they are not closed even when cellular displacements have deprived them of their former relations or functions. On the other hand, the fact that unpaired pits are not of more frequent occurrence seems to argue that Jost's assumption to the effect that displaced pits induce the formation of corresponding pits on contact with the walls of other cells, is probably often the case, except perhaps in instances where the unpitted walls encountered are too old. Neeff's observations also support such a view.

The tissue fusions resulting from budding and grafting also give support to the notion that pit connections that have been ruptured during gliding growth are subsequently resumed with the new neighbors on the completion of the process. However, it seems that actual contact with other tissues is not always necessary to induce the formation of pits, for many cases were found in proliferating tissues of apple and pear stems in which the proliferated cells were not in contact with other cells and yet contained pits. Vöchting[15] has made similar observations in Brassica.

Pits are often penetrated by very fine cytoplasmic threads that Strasburger[16] called Plasmodesmen. Such connecting threads have recently also been found to exist between the components of so-called graft hybrids.[17] Since cytoplasmic connections occur very generally between plant cells associated in tissues, it appears that they must frequently be ruptured in places where gliding growth occurs. That being the case, it seems unavoidable that thin cytoplasmic smears would result between the cells of such tissue, and that some intercellular spaces might be lined with thin films of such cytoplasm. According to Kny's[18] observations, published about ten years ago, intercellular cytoplasm is present in seeds of Lupinus. A little later,[19] however, he claims to have been in error in his first observations, in that the cytoplasm noted is said to have been carried to the intercellular spaces by the sectioning knife.

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[15] Vöchting, H. Untersuchungen zur experimentellen Anatomie und Pathologie des Pflanzenkörpers. Tübingen, 1908, pp. vii + 318, pls. 20.

[16] Strasburger, E. Ueber Plasmaverbindungen Pflanzlicher Zellen. Jahrb. Wiss. Bot. 36: 493–610. 1901.

[17] Hume, M. On the Presence of Connecting Threads in Graft Hybrids. New Phytologist 6: 216–221. 1913.

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[18] Kny, L. Studien über intercellulares Protoplasma. Ber. Deut. Bot. Gesellsch. **22**: 29–35; 347–55. 1904.

[19] ——— **23**: 96–98. 1905.

NOTES ON THE ANATOMY OF THE PUNCTATUS GALL

ALBAN STEWART

Our knowledge of the anatomy of Cynipid galls is based largely upon the investigations of Beyerinck, Hieronymus, Küstenmacher, Küster, Lacaze-Duthiers, Prillieux, and Weidel, but as the publications of these various authors have to do mainly with other than American galls, our knowledge of the anatomy of these remains meager for the most part.

During the summer of 1913 I was fortunate enough to discover a tree of the black oak, *Quercus velutina* Lam., in the western part of the state of Missouri, the smaller stems and branches of which were covered in places with galls caused by *Andricus punctatus* Bass. As there were galls on this tree in different stages of development, I collected a series and preserved them for further study. The galls caused by this insect are rather striking in appearance as they form knot-like swellings on the branches, which sometimes attain a diameter of five or more centimeters. The most of them, however, are smaller than this. They may occur singly, or two or more of them may be closely associated, one above the other on the same branch, which may become joined together by subsequent growth so as to appear as one gall. When they occur close together on the same branch, in the earlier stages of their development, the smaller galls are usually located towards the distal end.

The larger galls are roughened on the outside and are covered with small openings about 1 mm. in diameter, each of which leads into an empty larval chamber (fig. 10). Each opening is usually surrounded by a circular area, about 3 mm. in diameter, in which the surface of the bark is smoother and lighter in color than elsewhere. The openings persist for quite a while after the chambers are vacated, but they may ultimately become more or less obliterated by the subsequent growth of the gall. The larval chambers extend inward from the openings in a direction usually more or less perpendicular to the outer surface (fig. 2), so that cross sections of older galls will generally show various views of different chambers in the same section.

It is very evident that these galls may continue to grow for several years. The largest one in the collection has a cross-diameter of 6 cm. while the apparently normal stem just above it has a similar diameter of 1.6 cm. This portion of the stem has eight annual rings of growth, while the gall has but one ring of growth next to the pith which is normal, the rest of them being greatly altered in their structure. The second ring of growth from the pith shows less alteration than does the woody tissue outside of it. Its outer boundary is plainly marked, and, so far as one can judge by examination with a hand lens, it differs structurally from a normal ring of growth in having greater width and broader rays. The tissue outside of this shows no indication of annual rings except just inside the cambium where such a ring is differentiated in places. Another smaller gall examined has a diameter of 3.4 cm. and two apparently normal rings of growth at the center, while the stem just above it has a diameter of 8 mm. and five rings of growth. The tree from which the galls were taken has been infested with them for many years according to the inhabitants of the region. Similar galls are caused by this insect on *Quercus coccinea*, *Q. ilicifolia*, *Q. nigra*, and *Q. rubra* according to Cook (5, p. 28).

It is rather difficult to locate galls of this kind in their earliest stages as there is usually no external evidence of them beyond a slight swelling of the bark[1] such as might result from other causes. A cross section of an early stage is shown in fig. 1. The bark is about twice as thick on the side where the gall is located as it is on the opposite side of the stem, but there is practically no increase in the thickness of the wood. Apparently the first effect of the gall stimulus is to cause the formation of a larger amount of bark tissue than normal. Outside of the increase in the thickness of the bark no other striking abnormalities appear in the neighborhood of the gall.

The larval chamber is not as well defined as it is in later stages of development. The cavity containing the larva is irregular, conforming roughly in shape to it. The protecting layer of stone cells, somewhat lightly colored in fig. 1, is semilunar in outline and is only developed on the outside of the chamber in the section shown. Higher up in the same series of sections, however, a complete ring of stone cells has been formed. Just below the cavity, shown in fig. 1, there is a radial segment of brown-colored parenchyma cells which extends from the base of the cavity to a slight depression in the cork layers covering the gall on the outside. This segment is surrounded by several layers of cork cells, and it is possible that it has resulted from the sting of the insect in depositing the egg in the bark, as no other causal evidence is shown. Küster (13, p. 187) states, however, that wound-cork does not result from such action but callus tissue instead.

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[1] The term bark is used to include all tissues located outside the cambium.

There has been no increase in the formation of wood near the gall at this stage if a slight protuberance on the lower left hand side of the chamber in fig. 1 be excepted. In fact the production of woody tissue has been somewhat inhibited at the base of the chamber. A mass of parenchyma has been formed here, the cells of which have thin unlignified walls. Later the walls of these cells thicken and lignify resulting in the condition shown in fig. 3. A few short scalariform tracheids and other lignified elements are scattered among these parenchyma cells, very much disarranged in their positions.

In the stage of development shown in fig. 1, all of the chamber is surrounded by bark tissue except the inner side of it. A later stage (fig. 2) shows the inner part of a chamber to be embedded in woody tissue. The way in which this comes about can be studied to best advantage in serial sections cut tangentially through the gall. Usually the first woody tissue to appear, in the outermost sections of such a series, is small irregularly arranged masses, which, in sections a little further in, take roughly the form of small fibrovascular bundles (fig. 4). In this figure a small portion of the protecting layer is shown on the lower side. These pseudo-bundles sometimes join directly one with the other, or distinct ray-like masses of parenchyma and other cells may occur between them. The bundles often extend but part way around a chamber, other parts of it being surrounded by irregularly arranged masses of wood cells. Longitudinal sections through a chamber often show these bundles extending parallel with it in the barky covering of the gall. They are composed largely of bands of scalariform tracheids. Numerous galls have been examined in this regard and the bundles have always been found to be present to a greater or less extent. They are confined to the bark and thus form only the outer part of the woody covering of the chamber. The inner portion of the larval chamber is surrounded by woody tissue formed by the cambium. Soon after the gall starts, the cambium ceases its activity at the base of the chamber while it is stimulated to greater growth along the sides. The first stage of the enclosing process is shown in fig. 1, where a slight woody projection has formed at the lower left-hand side of the chamber. By further growth of the cambium a condition comes about like that shown in fig. 5, in which the wood has been formed well around the base of the chamber. The final result of this process is shown in fig. 2, where the inner third of the chamber is surrounded by wood. The enclosing method here outlined is somewhat similar to that described by Mäule (15, p. 24), where woody bodies, formed in the bark of wounded stems of *Salix phylicifolia*, are gradually surrounded by the activity of the cambium forming wood around them.

After a larval chamber is vacated by an insect the opening to the outside remains for some time. It is possible to stick a dissecting needle into the openings in some of the older galls for a distance of 1.5 cm. or more, and longitudinal sections through such chambers show that they may extend in nearly to the normal wood around the center which was evidently formed before the gall started. Sometimes the openings can still be seen several years after the cavity was vacated but after a time they may become closed and more or less obliterated by the growth of the bark around them.

A longitudinal section through a chamber recently vacated by an insect is shown in fig. 2. The chamber is lined with several layers of stone cells which, however, do not extend to the periphery of the gall. The thin-walled parenchyma cells, which fill the outer portion of the chamber before the insect emerges, have been largely destroyed in the section figured, and can only be seen to the right and left just inside the opening. In earlier stages, before the insect comes out, the stone cells extend inward from the periphery toward the center of the chamber and form a loosely arranged arch across it a short distance inside the opening. The loose arrangement of these cells offers but little resistance to the insect in leaving the chamber. Outside the protecting layer and running parallel with the sides there is a corky covering composed of several layers of cells, shown by a light line on each side of the chamber in fig. 2. It is probably somewhat similar to the corky covering around the protecting layer in the stem gall of the oak described by Lacaze-Duthiers (14, p. 338). It is not always the case that the protecting layer extends entirely around the chamber, as the inner end and sides of it may join directly onto the wood in these places.

If the protecting layer is examined in a longitudinal section through a chamber, it will be noticed that there are in general three kinds of stone cells which compose it. There are stone cells which are isodiametric or nearly so, and others which are much elongated in one direction, the last of which have their long axes arranged parallel with the chamber. There are still other stone cells which are more or less intermediate between the two kinds just mentioned, in which one diameter is somewhat longer than the other. Sections cut across a chamber sometimes show that these intermediate cells are arranged with their long axes perpendicular to it. It is evident that both isodiametric and elongated stone cells make up the protecting layer of this gall. There is a great difference in the thickening of the walls of the stone cells. Some have very thick walls with well-marked pits and small lumina, while others have thin walls and large lumina. Furthermore it is possible to find, among these, cells which have the walls unequally thickened on different sides, and which are very similar to those described by Weidel (21, pp. 329–330). Such cells do not occur in the normal parts of the oak, according to this author, but they do occur normally in certain members of the *Rosaceae* according to Küster (12, p. 183). Weidel found still further that stone cells with one sided thickening of their walls were confined largely to leaf galls in the forms studied by him. He also makes some interesting comparisons between the stone cells in Cynipid galls and those which occur normally in the oak. He finds that isodiametric stone cells are the ones that commonly occur in the normal parts, those which are elongated in one direction being confined to the bud scales and reproductive parts. It might be well to mention in this connection that Küster (12, p. 181) has found that the palisade-like stone cells with large lumina which occur in the gall of *Hormomyia fagi* call to mind similar cells that occur in the beech cupule. As elongated stone cells occur in the reproductive parts of the oak and related forms, one is led to suspect that possibly this kind of stone cell may be more primitive in the oak than the isodiametric form, since primitive characters are likely to persist in these parts. This supposition is strengthened by the fact that this kind of stone cell reappears in many oak galls so that the ability to form them must be latent and is only awakened to activity through the action of the gall stimulus. Such may be true of the ray structure to be considered further along in this article.

In young normal stems of this species of oak the stone cells of the bark are arranged in one or more concentric layers. Groups of stone cells may also occur opposite the broad rays, which are connected with one of the layers just mentioned. In the early stages of development of the gall this layered arrangement of the stone cells is not greatly interfered with, but in later stages the layers are broken up resulting in irregular masses scattered through the bark which are composed of stone cells larger than those normally present. The lumina of many of these contain a yellowish-brown substance which gives a strong reaction for tannin. They are evidently the same as the tannin balls described by Hartwich (10, pp. 147–149). Similar tannin accumulations also occur in other cells of the bark as well as in the cells of the protecting layer.

Corky inclusions occur quite commonly in the bark of the gall, which surround either groups of parenchyma or stone cells. One of these bodies is shown in fig. 7, where the cork forms a light colored circular figure at the center of the photograph. Mäule (15, p. 24) mentions cork growths in the bark of wounded stems but in these instances the cork is surrounded by a woody mantle composed of wood parenchyma cells and short tracheids. Groups of irregularly arranged tracheal elements also occur in the bark of the gall, the tracheids which compose them being short, sometimes nearly isodiametric, with scalariform markings on their walls. These may be arranged as single strands, or groups of tracheids may be associated together which are connected with similar groups a short distance away by cross bridging. The arrangement of the elements is often very complex resulting in twisted masses of tracheids as is shown in the center of fig. 6. Parenchyma cells sometimes occur in these tracheal complexes in the bark, the arrangement of which suggests narrow rays. Somewhat similar bodies are found in the bark of the black knot gall on *Prunus virginiana* (see Stewart 20, p. 120). Small groups of parenchyma cells may become partially surrounded by a woody growth in such a way as superficially to resemble the cross section of a sector of a small dicotyledonous stem, the parenchyma around which the woody growth takes place corresponding in position to the pith of such a stem. Some of these woody bodies have a cambium and seem to be capable of further growth.

But little is known about the origin of these peculiar woody bodies which occur in the bark. Mäule (15, p. 24) found special ball-formations[2] and other woody growths in the bark of wounded stems but was unable to explain their origin. It is evident that he searched carefully for the beginning stages of such but did not succeed in finding them. Krick (11, p. 17) has also found somewhat similar bodies to be normally present in the bark of the red beech, some of which had cork for a central nucleus while still others had wood. It is possible that many of these woody complexes in the bark may originate from misplaced cambium cells. Such is evidently the case in the galls caused by the black knot fungus on the choke-cherry (Stewart 20, pp. 119–120).

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[2] The term "Knäuel," used in the German literature to designate these peculiar woody growths, is rather difficult properly to express in English. Miss Frances

With the possible exception of the pine, it is likely that the structure of no wood is better known generally than that of the oak, as both of these woods are usually studied in elementary courses in plant histology in institutions where such courses are offered. The results of a large amount of investigation have been published on the structure of oak wood but to attempt to enumerate all of these would be superfluous in this article. Hartig (9, pp. 92–96) gives a rather detailed account of the structure of this wood, and Bailey (2 and 3), Bailey and Sinnott (4), and Eames (7), treat the ray structure rather exhaustively.

Normal oak wood is composed of vessels, tracheids, fibers, and parenchyma cells, the last of which occur both as wood and ray parenchyma. The vessels are very large and there is a tendency for them to form scalariform perforations according to Solereder (19, p. 781). In some species, especially those belonging to the white oak group (subgenus Lepidobalanus), the vessels become filled with tyloses after a time. Gerry (8, pp. 451, 455–456) has recently done some work on the subject of tyloses and finds that in contrast with the white oaks the members of the red-oak group (subgenus Erythrobalanus) are normally free from tyloses with many exceptions. Tyloses occur scattered or frequent in the sap wood of *Quercus velutina* and scattered in the heart wood. In the closely related scarlet oak *Q. coccinea* they are usually scattered but may be abundant in connection with fungus growths. The formation of tyloses is easily induced through the wound stimulus. Gerry (8, p. 447) found that even the wound produced by felling the tree may cause the formation of tyloses in the outer rings of *Q. michauxii.*

Dorrance, who is completing a translation of Küster's Pathologische Pflanzenanatomie, has been kind enough to supply me with some of her translations of this and closely related terms. Among these are: knarl, ball, and ball-formation.

Specimens of a number of the normal branches were taken from the tree from which the galls were collected to be used in comparing the normal wood with that of the gall. Without exception these specimens show tyloses to be present in the vessels, and their abundance in places leads one to suspect that they may not all have been normally produced. Possibly the gall stimulus has had something to do with their production. I have been unable to find any references in the literature on the anatomy of galls which records exactly similar conditions, but it seems to be an established fact that the stimulus resulting from parasitic fungi is capable of exerting an influence upon the structure of the host plant some distance beyond where the fungus actually occurs. The normal wood of *Abies* contains no resin canals, but Anderson (1, p. 465) found them to be present in all branches above parts infected with *Aecidium elatinum*. He also found (pp. 473, 476) that the branches of *Pinus Strobus*, *Larix japonica*, and *Picea excelsa*, which were situated above parts infected with *Agaricus melleus*, contained a greater number of resin canals than did the normal wood of these species. Mer (16, p. 367) also states that resin canals occur in the branches of *Abies pectinata* which are above parts infected with *Phoma abietinae*. Rather striking abnormalities may result from the wound stimulus some distance away from the actual wound. Mäule (15, p. 12) found in *Rosa centifolia* that very broad rays and short wood cells persist 5–6 cm. from the wound, and De Vries (6, p. 20) found evidences of wounding in the structure of the wood of the *Caragana aborescens* 7 cm. from the wound. The above citations give sufficient ground for believing that if the tyloses in the branches of this oak result abnormally, the gall stimulus is the cause of it.

The origin and structure of the broad rays in oak wood has been the subject of much study and discussion in recent years. As is well known, the rays in oak wood are of two kinds: those which are one cell wide in cross and tangential section, known as uniseriate rays, and those which are more than one cell wide. The rays of the second group can be further subdivided into those which are 2–3 cells wide, multiseriate, and broad homogeneous plates of parenchyma tissue, or compound rays. Of these three kinds, the uniseriate rays are usually considered to be the more primitive.

Until rather recently the broad rays, the so-called primary medullary rays, have been considered to be inclusions of the fundamental tissue between the primary fibrovascular bundles. In his study of the structure of the wood of fossil oaks from the Miocene of California Eames (7, pp. 161–163) found uniseriate rays in his sections, but none of the broad homogeneous rays, their place being taken by groups of narrow rays aggregated together. Eames also studied the wood structure of a number of seedling oaks and found in the group of black oaks, to which *Q. velutina* belongs, that the first broad rays formed were similar in structure to those in the fossil oaks. He found still further that the homogeneous rays come about through a loss of the fibers, etc., in the aggregations of narrower rays, so that the compound rays of adult wood are formed after a time by this means. In the group of white oaks he found a still more primitive condition in the seedling as only uniseriate rays occur for a foot or more above the ground and extending through 15–20 annual rings of growth. The broad rays of the mature wood arise by aggregation and fusion of the narrow rays in the manner just stated.

Our knowledge of the structure of the oak ray was still further advanced by the work of Bailey (2), who found in his studies of traumatic oak wood, that the rays formed immediately after wounding were all uniseriate. By aggregation and fusion of these the normal compound rays result again in subsequent layers. His results seem to show that the ancestral conditions of ray structure are possibly recapitulated in wounded areas. In a later paper, however, Bailey and Sinnott (4) 46, state that seedling stems and roots of *Quercus alba*, which possess only uniseriate rays, will form wide multiseriate rays after injury, often without indication of the putative stages of compounding.

In the examination of tangential sections of this gall I found rays which were similar in many respects to those figured by Bailey (2, pl. XI, fig. 2). In fact a large number of the broad rays in different galls showed an inclusion of fibers and other elements to a greater or less extent, but usually not so strongly marked as in the ray figured by Bailey. This condition led to the supposition that possibly a repetition of the conditions described by Eames and Bailey might take place in their formation. In order to determine whether this supposition was true or not, one of the smaller galls was selected, and tangential sections cut of it from the outermost layers of gall wood to the normal wood inside, formed before the gall started. A larval chamber was located on this side of the gall, and the section were cut at right angles to its long diameter as shown in fig. 2. Every other section was mounted in glycerine and preserved in series for study.

The first rays to appear in the outermost sections of this series are narrow for the most part, usually 1–4 cells wide. There are occasional rays, however, which are somewhat wider but none so wide as those which occur in sections taken farther in. A little deeper in the gall wood many of the rays broaden and occasionally there is a very wide one in which there are inclusions of fibers. Some of the ray combinations in this region are quite similar to the traumatic ray of *Quercus nigra* figured by Bailey (*l. c.*). Each succeeding group of a few sections towards the interior of the gall shows a broader zone of wood around the larval chamber. With the increase in the amount of wood, many of the rays also increase in width until they reach the size shown in fig. 8. Some of these rays have inclusions of fibers as is shown in the figure, while others are practically free from such. Uniseriate rays also occur here among the fibers, etc., which lie between the broad rays.

By examination of sections still deeper in the gall from those shown in fig. 8, a condition is finally reached in which the rays are practically all uniseriate. This condition is shown in fig. 9, which was taken from a part of a section corresponding closely in position with that shown in fig. 8. It is further evident that the section shown in fig. 9, was of gall wood and not of normal wood, which occurs a short distance inside, because there is a parenchymatous area to the right, not shown in the photograph, which was located near the base of the larval chamber. It is similar in structure to the part shown in fig. 3 which was taken from near the base of the larval chamber of this same gall. The section from which fig. 9 was taken also includes some of the stem just below the gall, a portion of which is shown in fig. 11. The structure of the wood is nearly normal in this portion and it is interesting to follow the broad rays from the part of the section shown in this figure, to the part shown in fig. 9, as they gradually break up into smaller ones by fiber inclusions as the section through the gall is approached.

We have here a rather striking correlation between the effects produced by the gall stimulus and those which come about after wounding, as the results are very much the same in each case. There is this difference, however, that after the formation of the broad rays, they may break up or be replaced by narrower ones farther out in the gall. If such a condition exists in traumatic oak wood it is not mentioned by Bailey. The reversion to a supposedly primitive ray structure in each instance is rather striking.

There are still other interesting things to be noticed in the ray structure of this gall. The broad rays, mentioned in the preceding paragraph, are more or less fusiform as a rule when seen in tangential section, and are much shorter vertically than in normal wood. De Vries (6, p. 83) also found a vertical shortening of the rays in primary wound wood. Between the pseudo-fibrovascular bundles, which form the outer part of the woody covering around the larval chambers, there are often ray-like structures present which are composed of several layers of unlignified parenchyma cells (fig. 4) radially arranged with reference to the chambers. Raylike bodies sometimes appear between these bundles, which on closer examination are seen to be composed of short scalariform tracheids turned over so that their long axes are presented. There are often large areas within the gall composed of ray-like cells but which are too irregular in shape to be considered as rays (fig. 3). Somewhat similar areas occur in traumatic wood of this species of oak.

From what has been said concerning the ray structure in the preceding paragraphs one would conclude that the course of the fibers and other elements would differ greatly from normal in the wood of the gall, and such is the case. In places where broad rays occur the fibers and other cells of the wood are very much bent from their usual upright course to conform to the shape of the rays. Individual strands of fibers or groups of them often pursue a very irregular course through them (fig. 8). In places where there are a number of closely associated multiseriate rays the course of the fibers surrounding them is often wavy. The complexity of fiber arrangement is often so great as to result in true ball formations as is shown in fig. 13. Mäule (15, p. 9) has done some work on these peculiar fiber arrangements and concludes that they come about to a great extent through the attempt of the young wood cells to reach their normal length when they meet with resistance in their growth. According to Mäule the young wood cells can bend in three directions, either outward radially, or to right and left tangentially. They never bend inward because the older wood lies in this direction. Their usual course of bending is to right and left so that they are generally seen in tangential sections. They occur but seldom in this gall but when they do appear they are always in tangential sections.

There is a great disturbance in the course of the fibers around the larval chambers and around the masses of lignified parenchyma cells which occur near the bases of such. There is often a parting of the fibers to right and left tangentially in such regions, a condition which is still apparent for some distance above and below a chamber. Mäule (15, pp. 19–20, pl. 1, fig. 8) describes and figures a somewhat similar arrangement of fibers in the wood formed in a direct line with longitudinal wounds on stems of *Cornus sibirica*. The structure of the masses of lignified parenchyma, which occur around the bases of the larval chambers (fig. 3) are very complex and no regular arrangement of the cells can be distinguished. The cells in such places are mostly short and are not dissimilar to the short-celled elements which are formed immediately after wounding. Fibers are sometimes mixed with the parenchyma cells in which case they pursue a very irregular course. Mäule (15, p. 7) mentions conditions somewhat similar to this near wounds on stems of *Caragana*, *Abies*, *Aesculus*, *Salix*, and *Tilia*.

The course of the cells in the wood appears to be nearly normal in most places in cross sections of galls as the greater part of them are not so greatly bent from their upright course as to be readily apparent in such sections. There are places, however, in which the course of the cells is very much altered, and small segments of the wood may be turned over nearly at right angles so that the long axes of the cells are presented. Such an arrangement is shown in the lower right hand side of fig. 14. In places of this kind the shortness of the cells which are turned over is very apparent. The change in direction of the cells from vertical to horizontal or nearly so, is usually sudden and there are seldom pronounced transitional stages between. Cross sections also show a great reduction in the number of vessels, and where the disturbance has been greatest, an entire absence of them. They occur more commonly in the gall wood first formed, from which narrow vessel-containing segments may extend outward into wood from which they are otherwise absent (fig. 12). Sometimes the narrow vessel-containing segments extend nearly to the cambium. In traumatic wood of these species of oak, formed soon after wounding, there is a great reduction in the number or an entire lack of vessels.

In cross sections of many of the older galls a layer of wood has been formed just inside the cambium which is normal in many respects (figs. 5 and 14). This layer sometimes extends through nearly the entire circumference of the gall, but more often only segments of it occur. The cells in this layer usually have nearly their normal arrangement and associations. Large vessels appear which do not occur in the wood just inside of it. Occasionally small segments of this layer are turned over showing that the cells which compose it are shorter than normal. The layer as a whole is as sharply set off from the wood inside as are two annual rings of growth. It looks very much as if there is here a suggestion of a return again to normal growth. It is a well known fact that after a time the cambium returns to its normal functions in wounded areas, after the wound is healed.

In radial sections through galls the most of the tissue presented is ray tissue. Owing to the fact that so many of the fibers are bent to right and left tangentially, but few entire ones appear in such sections, as the most of them have been cut across. One often finds in the vicinity of larval chambers in these sections what appears to be a nearly true cross-section structure of wood (see lower left hand side of fig. 2). Such areas are made up mostly of fibers.

Tannin bodies, similar to those which occur in the stone and other cells of the bark, also occur in many of the cells of the wood. These bodies may completely fill the lumina of the cells or only partly so. They are either homogeneous or granular in appearance.

In concluding this article it seems desirable to summarize briefly the important facts contained in it. The presence of elongated stone cells in the protecting layer around the larval chambers is a fact of some interest as these cells occur normally in no others part of the oak than the bud scales, and in the reproductive parts, the last of which is an important place for the retention of primitive characters. The presence of stone cells in the protecting layer with unequally thickened walls is another fact of interest, as such cells do not occur normally in the oak. They are usually confined in their distribution to the leaf galls on it.

The following conditions in this gall can be correlated with similar conditions in traumatic tissue:

1. A recapitulation of similar conditions of ray structure.

2. A vertical shortening of the broad rays.

3. The presence of ball-formations in the wood, which appear only in tangential sections.

4. A parting of the fibers in the vicinity of the larval chambers, similar to the condition resulting from longitudinal wounds.

5. Isodiametric parenchyma cells around the base of the larval chambers with irregularly distributed fibers and other woody elements among them.

A great reduction in the number, or an entire lack of vessels.

7. A shortening of many of the cells of the wood.

8. Absence of distinct annual rings of growth.

9. A suggestion of the return of the cambium to normal activity after a time.

10. Woody inclusions in the bark.

The fact that similarities often exist between the structure of galls and traumatic tissue is well known among workers in pathological plant anatomy. Küster (13) mentions this fact in several places in his work. Possibly one of the most noteworthy single examples of this kind is that mentioned by Molliard (17, p. 200) in which by pinching the buds of *Pelargonium zonale* structures were produced similar to those in the flower galls on *Geranium dissectum* caused by *Cecidophyes Schlechtendali.* I believe, however, that this is the first time that the conditions in a single gall have been correlated with the conditions in traumatic tissue in so many important ways. The comparisons made with wound tissue in this article have been based largely upon the general results of De Vries and Mäule. I hope, however, to continue further this investigation on other woody oak galls, and to compare more closely the structure of the gall wood with that of traumatic wood of the oak.

METHODS

The galls used for sectioning in this work were preserved in a weak solution of formaldehyde when they were collected and left in it until they could be further treated. In order to prepare sections for study, the galls were first macerated to remove the formaldehyde, and were then placed under the receiver of an air-pump and the air exhausted until bubbles ceased to rise from them. They were then transferred to strong hydrofluoric acid and left for about two months in order to remove all of the mineral matter. No difficulty was experienced in cutting sections less than 10 μ in thickness except that large sections, cut thinner than this, were badly broken up in the subsequent handling. Sections 10 μ in thickness were used in most instances as they were found to be sufficiently thin for all that was required.

An alcoholic solution of safranin was employed for staining, and as a counter stain "licht Grün" dissolved in clove oil was used. This had the double advantage of both counterstaining and clearing the sections. Iron-alum haematoxylin was tried as a counter stain, but owing to the large amount of tannin in the galls, it was not a success. Where serial sections were necessary they were cut somewhat thicker as a rule and mounted in glycerine. Such of these as were required for close study or photomicrographing were removed from the series, stained, and mounted separately.

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Department of Botany, University of Wisconsin,
Madison.

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DESCRIPTION OF FIGURES IN PLATES LI AND LII

All figures are of the Punctatus gall from *Quercus velutina.*

Plate LI

Fig. 1. Cross section of a very young gall showing larval chamber. × 13.

Fig. 2. Longitudinal section through a larval chamber shortly after it has been vacated by the insect. × 9.

Fig. 3. Tangential section through an old gall showing a portion of the complex of isodiametric parenchyma cells near the base of the larval chamber. × 33.

Fig. 4. Tangential section showing pseudo-fibrovascular bundles around a portion of a larval chamber. A part of the protecting layer is shown on the lower side of the figure. × 33.

Fig. 5. Tangential section through a gall showing a larval chamber being enclosed by woody tissue. Intermediate between the stages shown in figs. 1 and 2. × 9.

Fig. 6. Woody inclusion in the bark of the gall. Light colored body at center of figure. × 33.

Fig. 7. A cork inclusion in the bark of the gall. Light area surrounding the center. × 33.

Plate LII

Fig. 8. Tangential section through outer woody covering of gall showing uniseriate and compound rays. × 33.

Fig. 9. Tangential section through central portion of gall showing only narrow, mostly uniseriate rays. × 33.

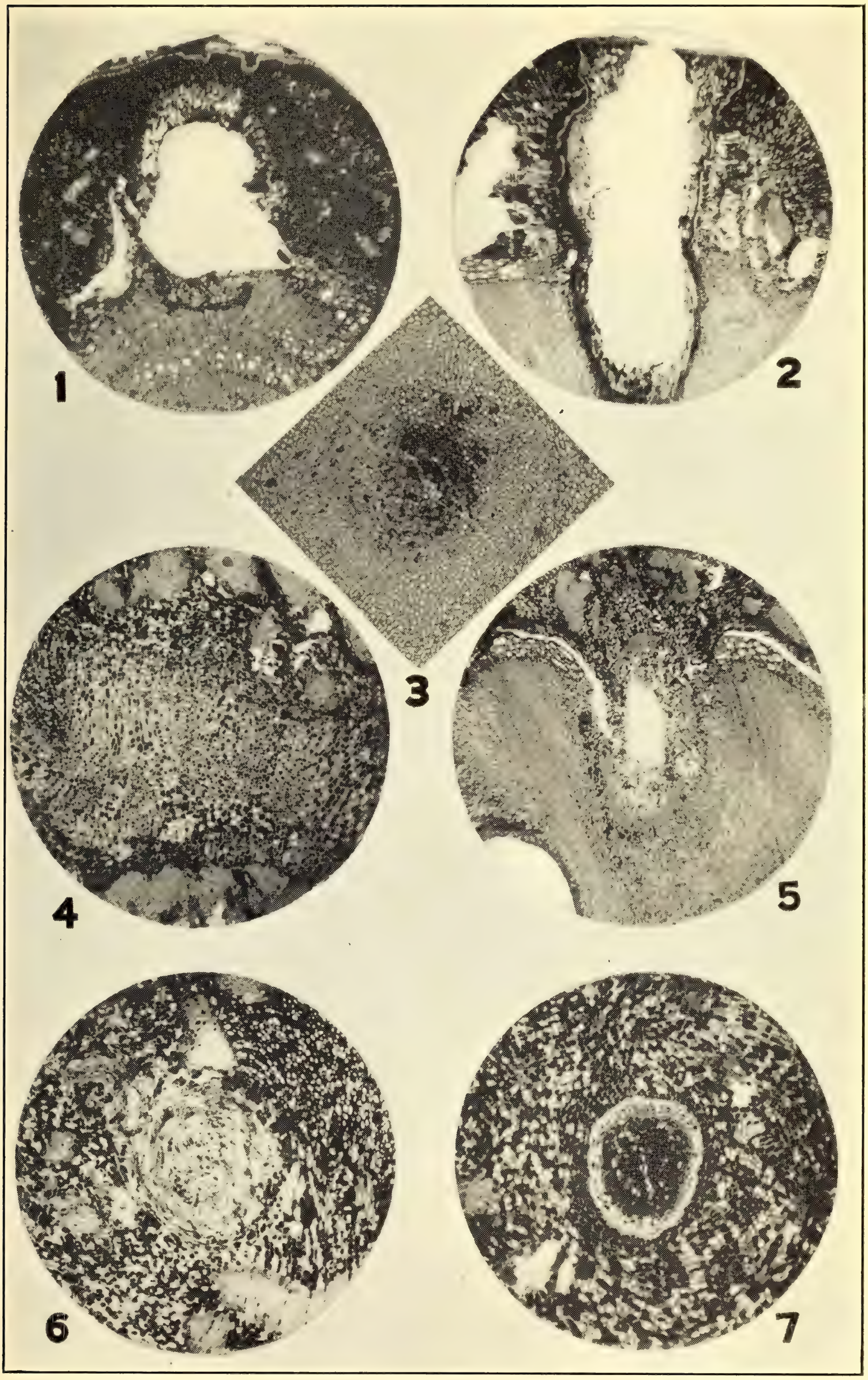
Fig. 10. External view of the punctatus gall. Openings to chambers shown by dark dots. × about ½.

Fig. 11. Tangential section through a portion of the stem below gall showing nearly normal ray structure. Photograph taken from same section as part shown in figure 9. × 33.

Fig. 12. Cross section of a portion of a gall showing radial sector containing vessels in the wood of the gall. × 33.

Fig. 13. Tangential section showing ball-formation (Knäuel) in wood of gall. × 33.

Fig. 14. Cross section of a portion of an old gall showing apparent return to normal growth. The light line across the figure is caused by a slight separation of the wood and bark at the cambium. × 33.



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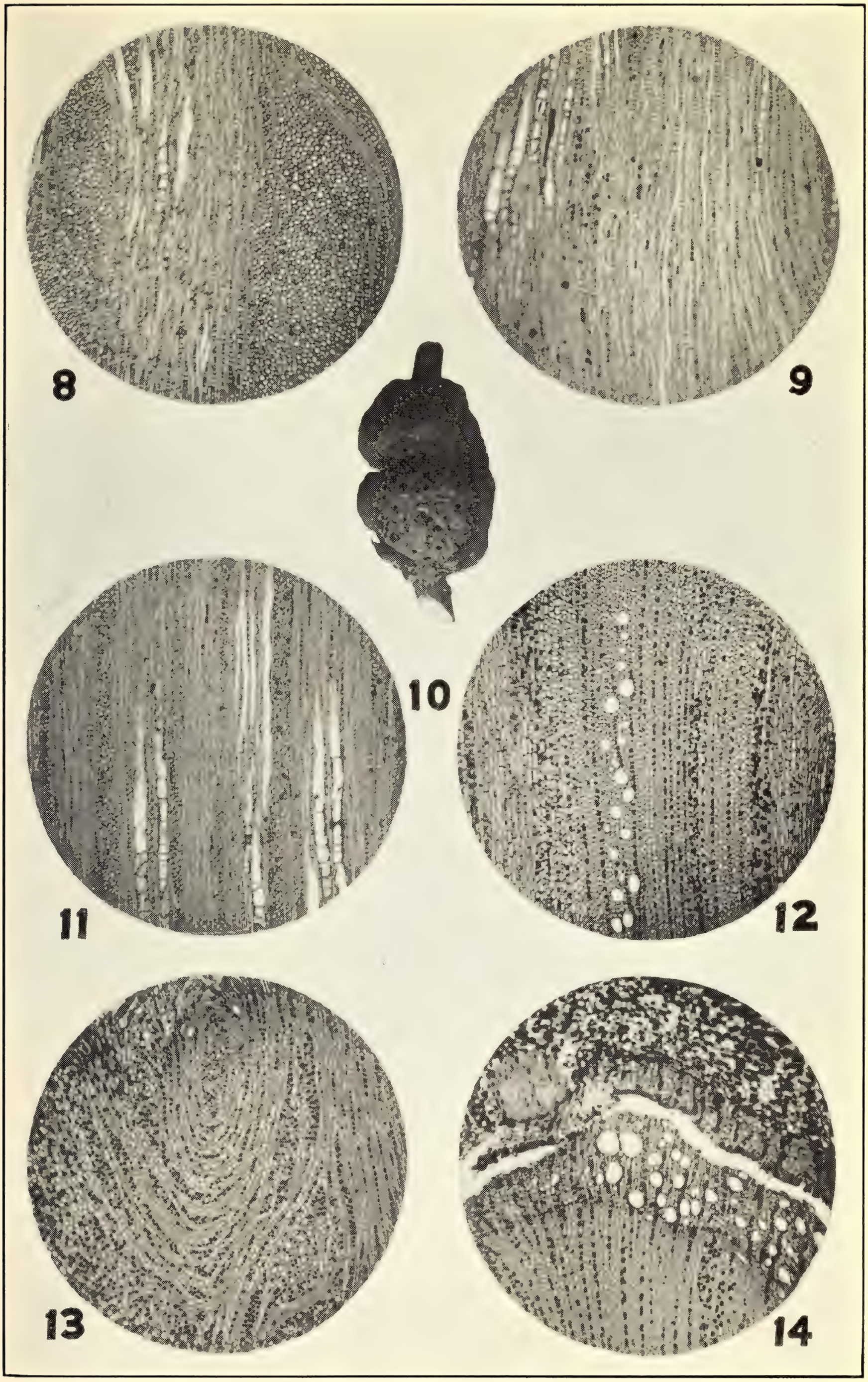
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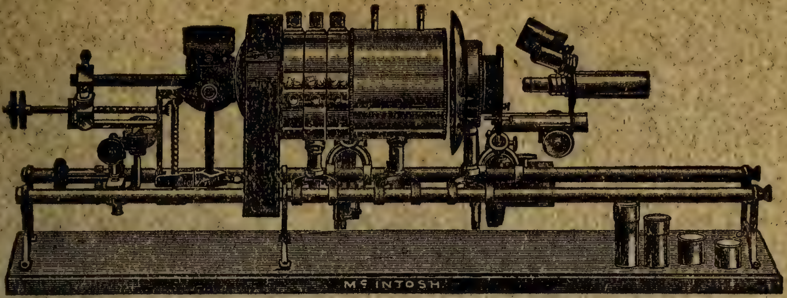

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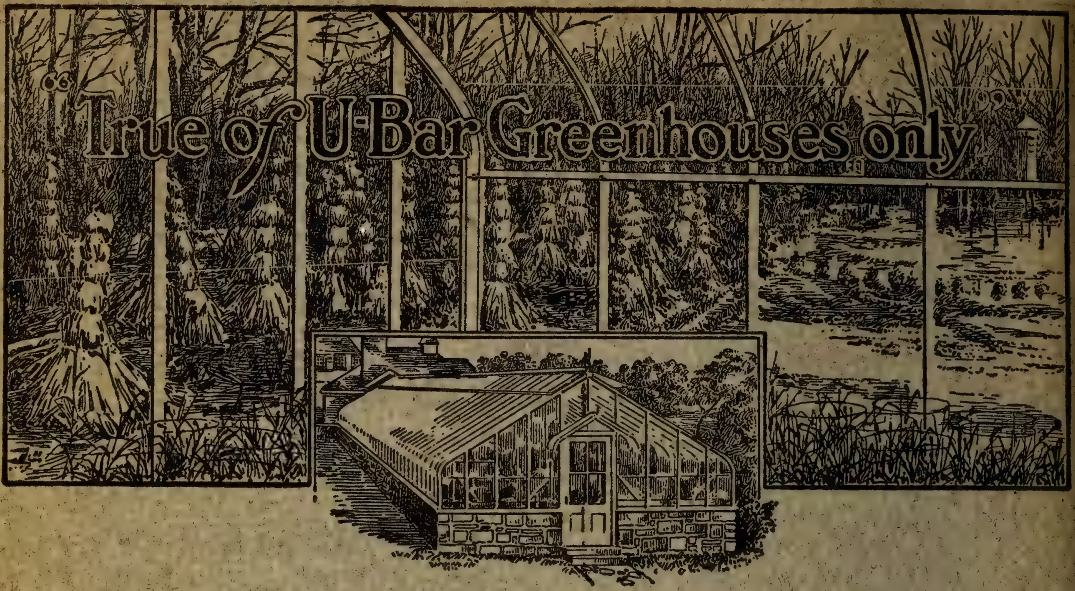
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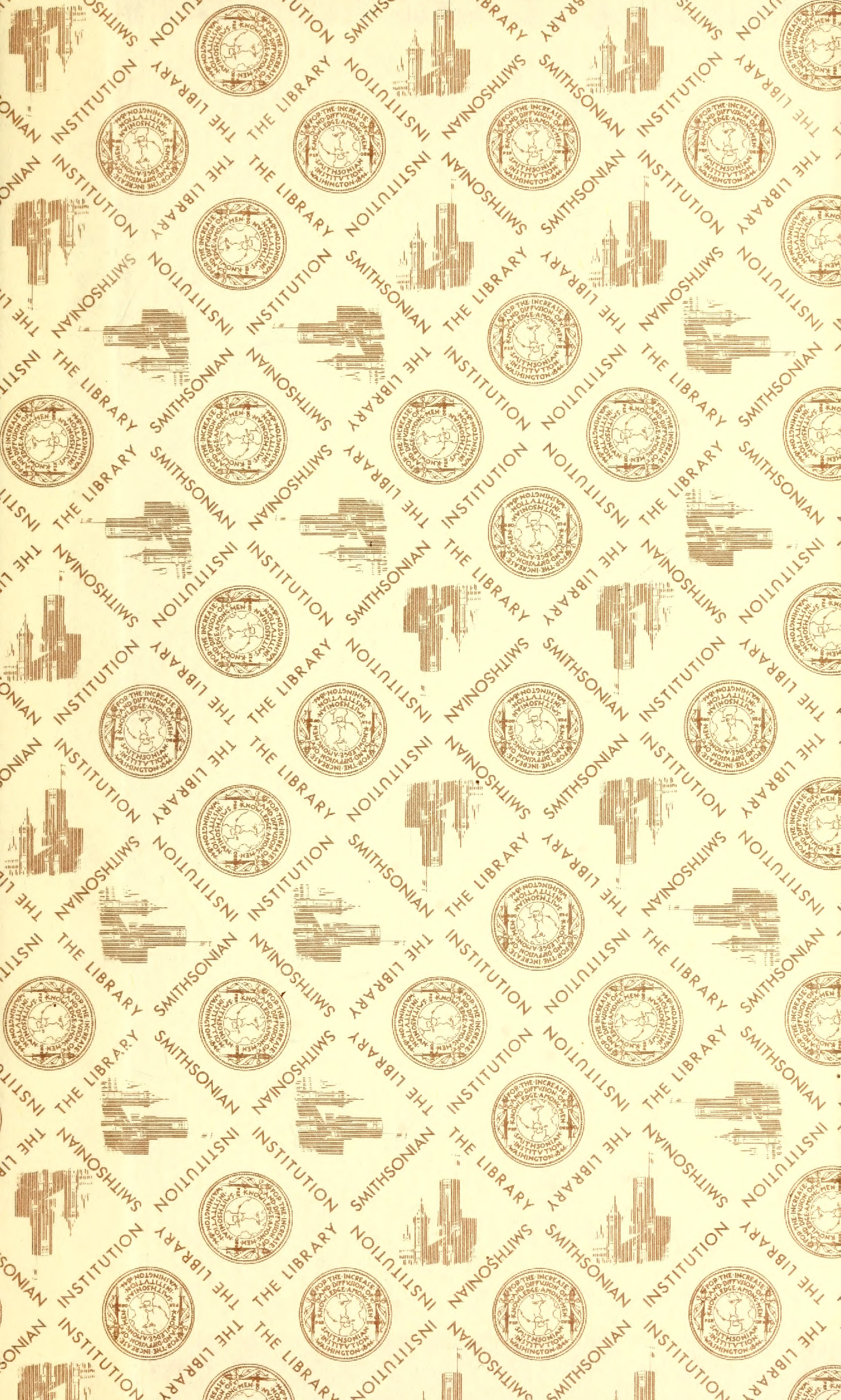
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